THE EFFECTS OF SUPPLEMENTATION OF β-CAROTENE DURING THE CLOSE-UP PERIOD ON COWS, COLOSTRUM, AND CALVES

BY

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THESIS

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ABSTRACT

Due to consumer demands, the dairy industry needs to investigate ways to keep dairy cattle healthy, productive, and profitable while minimizing the use of drugs. One way to accomplish this may be by inclusion of antioxidants in the diet. An important antioxidant that should be considered is β-carotene. β-Carotene is found naturally in many plants and is a dual-purpose nutrient, having both provitamin A and antioxidative functions. Ingested β-carotene can be cleaved into two molecules of retinol if the animal is in need of retinoids. If vitamin A stores are sufficient, β-carotene will be used to help dispose of potentially harmful reactive oxygen species. Reducing oxidative stress may help cows restore their positive energy balance following parturition, as well as possibly decrease pneumonia in young calves. Our objective for this study was to determine the effects of β-carotene supplementation on the cow, her colostrum, and her calf. The trial was conducted on a large, commercial dairy farm in northern Indiana. Ninety-four multiparous Holstein cows were assigned to either a treatment or control group. Each cow individually received a concentrate mix topdressed on to her TMR while in a headlock each morning beginning 21 d prior to expected calving and ending at calving. The treatment group was supplemented with 8 g of Rovimix (800 mg of β-carotene) in the topdress. Body condition score was taken on day of enrollment (d -21) and upon trial completion (d 7). Blood samples were obtained from cows on d -21, -7, 0, and 7 relative to calving. All samples were protected from light. Whole blood samples were analyzed for β-carotene using an iCheck (BioAnalyt; Teltow, Germany) immediately following collection. Serum samples were frozen at -20ºC for later analysis. Similarly, blood samples were collected from the calves at d 0, 1, 7, and 60. Samples were immediately analyzed for concentrations of β-carotene and total protein prior to the serum being frozen. Calves were fed 3.78 L of dam-specific colostrum and colostrum was sampled immediately following parturition. Fresh samples were used for immediate BRIX and

β-carotene analysis, as well as for component and colorimeter analysis. Feed samples were collected weekly throughout the trial and nutrient composition of forage and TMR samples was determined. Health records for the animals were collected from DairyComp305. The farm staff was responsible for all care of the animals. Colostrum, serum, and feed samples were analyzed for concentrations of vitamin A, vitamin E, and β-carotene. A full metabolite profile was determined in serum from cows and calves. Proc Mixed, Proc Glimmix, and Proc Freq, among others, in SAS 9.4 were used to analyze the collected data. The rations were adequate for vitamin A, with concentrations in the TMR exceeding NRC requirements by 20% and 276% for close-up and fresh diets, respectively. Vitamin E in the TMR was just under requirements at 92.2% and 93.0% of NRC requirements for close-up and fresh diets, respectively. β-Carotene supplementation significantly increased ($P = 0.023$) serum concentrations of vitamin A in cows, indicating that the high amounts of supplemented vitamin A in the diet were still not enough to release β-carotene from its provitamin A role. Serum vitamin E concentrations were not affected, indicating that there was no interaction between it and β-carotene. Serum β-carotene concentrations were significantly greater ($P < 0.01$) for the treatment group on all days when compared with the control group. The concentration of TP was higher ($P = 0.045$) in β-carotene supplemented cows. β-Carotene supplementation also decreased the concentration of albumin ($P = 0.029$), increased the concentration of globulin ($P <0.01$), and affected the ratio between the two ($P < 0.01$), but these results were confounded by a significant or trending interaction of treatment and parity. No significant effects were detected in reproductive, health, or milk yield variables extracted from DairyComp305. Supplementation of β-carotene increased the concentrations of β-carotene ($P < 0.01$) and fat ($P = 0.042$) in colostrum. It also increased the colorimetric values for a* ($P = 0.014$) and b* ($P< 0.01$), which indicates that the β-carotene-rich

colostrum was significantly more red-yellow in color than the colostrum from control cows. The effects of β-carotene supplementation to the dam were negligible in calves. There were no differences in the concentrations of vitamins A and E in calf serum. Significant effects or trends were observed for concentrations of gamma-glutamyl transferase ($P < 0.01$), blood urea nitrogen ($P = 0.044$), β-hydroxybutyrate ($P = 0.097$), and phosphorus ($P = 0.088$), but, with the exception of phosphorus, these results were confounded by significant or trending treatment by parity interactions. There was also a significant interaction of treatment by time for gamma-glutamyltransferase ($P < 0.01$). The majority of calf serum samples had β-carotene below detectable levels. Because of this, Proc Freq was used to determine if there was a treatment difference in the number of calves above or below the detection threshold of 0.05 μg/mL. There were 28 samples above the threshold at 24 h of age, with 89.3% ($P < 0.01$) of the calves with detectable β-carotene concentrations being from β-carotene-supplemented dams. At d 7, there were only 7 calves with detectable concentrations. Of the 7 calves, 85.7% ($P = 0.045$) were from β-carotene-supplemented dams. Only one sample at d 0 had detectable β-carotene concentration and none did at d 60. This fleeting response shows that supplementing the dam with β-carotene does not substantially affect the calf and direct β-carotene supplementation to the calf should be considered.

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ABBREVIATIONS

AST	aspartate aminotransferase
βC	β-carotene
BCS	body condition score
BHBA	β-hydroxybutyric acid
BUN	blood urea nitrogen
BW	bodyweight
Ca	calcium
CL	confidence limit
CP	crude protein
CPK	creatine phosphokinase
d	day(s)
DA	displaced abomasum
DIM	days in milk
dL	deciliter(s)
DM	dry matter
g	gram(s)
GGT	gamma-glutamyl transpeptidase
GLDH	glutamate dehydrogenase
GSH-Px	glutathione peroxidase
h	hour(s)
HPLC	high performance liquid chromatography
Ig	immunogloblulins
IU	international unit
K	potassium

kg	kilogram(s)
L	liter(s)
LDL	low density lipoprotein
LPO	lipid peroxides
LS	least squares
Mcal	megacalorie
mEq	milliequivalent
Mg	magnesium
mg	milligram(s)
mL	milliliter(s)
mmol	millimole(s)
MUN	milk urea nitrogen
Na	sodium
NEFA	non-esterified fatty acid(s)
ng	nanogram(s)
OR	odds ratio
P	phosphorus
RAR	retinoic acid receptor
ROS	reactive oxygen species
RP	retained placenta
RXR	retinoid X receptor
SCC	somatic cell count
SD	standard deviation
SE	standard error
SOD	superoxide dismutase

TBARS	thiobarbituric acid reactive substances
TMR	total mixed ration
TP	total protein
U	unit(s)
μg	microgram(s)
Vit	vitamin
wk	week(s)

CHAPTER I: LITERATURE REVIEW

Transition Period

It is rather easy to argue that the transition period, which is defined as the 3 wk prepartum and 3 wk postpartum, is the most crucial time period for a modern dairy cow (Drackley, 1999). During the last 60 d prior to calving, generally the dry period for the cow, the fetus gains approximately 60% of its birth weight (Bauman and Currie, 1980). This drastic weight gain by the calf places a heavy metabolic burden on the dam. At the same time, it is well accepted that the cow's feed intake decreases as parturition nears, which only further tips the scale in the direction of a negative energy balance. The metabolic burden of the developing calf, coupled with the waning feed consumption, will most likely force the cow to undertake necessary biological steps to adapt metabolism to meet her energy needs.

Even more than the demands of the fetus, nutrients are also required by the cow to support their developing mammary system, production of colostrum, and initiation of milk synthesis. Similarly to meeting the fetal needs, the cow will break down adipose tissue and glycogen reserves, upregulate gluconeogenesis, mobilize protein stores from the muscle and other tissue, and mobilize calcium from the skeletal system in preparation for milk synthesis (Bauman and Currie, 1980). Because the volume of milk produced steadily increases until about 45 to 90 DIM, the cow may need several weeks to restore her energy balance.

If the cow isn't able to quickly and effectively address her nutrient needs following calving, there may be an occurrence of metabolic and reproductive disorders such as fatty liver, ketosis, hypocalcemia, displaced abomasum, and retained placenta. An excess of mobilized lipids can especially put the animal at risk for these issues (Drackley, 1999). Mobilization of triglycerides is often represented by the amount of non-esterified fatty acids (NEFA) in the

blood. Periods of high NEFA concentrations, as well as decreased feed intake, put the cow at risk for both fatty liver and ketosis (Bertics et al., 1992; Grummer, 1993). Similarly, the level of β-hydroxybutyric acid (BHBA) in circulation is commonly used as a measure of ketone production, with high levels being associated with ketosis. Increased amounts of NEFA and BHBA are thus indicative of an insufficient amount of energy relative to metabolic need.

Goff and Horst (1997) listed three keys for the cow to avoid disease following calving. These keys are 1) successfully adapting from the low energy prepartum diets to high energy postpartum diets, 2) sustaining proper blood calcium concentrations, and 3) maintaining a robust immune defense. Anything the producer can do to assist the cow in meeting these goals will help the animal be healthy and productive throughout the transition period, the remainder of the lactation, and hopefully many lactations to come.

Colostrum

The first mammary secretion that the cow produces following parturition is referred to as the colostrum. The role of colostrum for the neonate is two-fold: (1) provide passive immunity transfer from dam to calf, and (2) provide nutrients. One of the unique factors of colostrum is the high amount of immunoglobulins, especially immunoglobulin G (IgG). The transfer of these IgG from the dam's blood to the colostrum and then to the calf is critical for the neonate, because it has little to no protection from its naïve immune system. Having just departed the safety of the placenta, the calf enters a novel, relatively dangerous environment. The calf is able to absorb the IgG from the colostrum for approximately 2 d following birth, with the amount absorbed diminishing rapidly after the first few hours of life. Przybylska et al. (2007) stress that the first 24 h of life are the most important for neonates because poor colostrum quality, insufficient

colostrum volume ingested (< 2 L), or issues with intestinal permeability can all cause inadequate immunity, which can lead to morbidity and mortality.

Additionally, colostrum contains many nutrients to benefit the calf. Because little placental transfer of immunoglubulins and some vitamins occurs in bovines, the colostrum is crucial for meeting the calf's initial needs for these nutrients (Quigley and Drewry, 1998; Frandson, 2003). Colostrum also includes water, lipids, carbohydrates, proteins, and minerals, which are also needed for the calf to sustain life. The fats and carbohydrates are used as an energy source by the calf, which is especially important in cold weather, while the non-IgG proteins are digested to amino acids, which are absorbed and used for protein synthesis (Quigley and Drewry, 1998). The vitamins contained in colostrum will be discussed in detail later.

Lastly, colostrum contains many important antioxidants, which are less frequently mentioned. Some known ones are lactoperoxidase, superoxide dismutase, catalase, glutathione peroxidase, lactoferrin, vitamin C, vitamin E, and carotenoids (Lindmark-Mansson and Akesson, 2000; Przybylska et al., 2007). The importance of antioxidants will be examined in a later section. A study by Yang et al. (2015) looked at many of the aforementioned nutrients and antioxidants in colostrum and found that calves fed colostrum at birth had higher serum concentrations of total protein, IgG, and superoxide dismutase, as well as increased weight gain and enhanced intestinal development, compared to calves that received regular milk. All in all, as Albera and Kankofer (2009) summarized so succinctly, "[f]or newborns, colostrum contents should cover all aspects of protection against new environment".

Calves

As colostrum has unique qualities, so does the neonatal calf; the chief one being that for the first few weeks of life, they are considered to be pre-ruminants. During that period, their

rumen is still developing so their digestive system begins as one more similar to a nonruminant animal than to the older animals of their own species. Because of these changes, the first months of life of the calf have been broken into three categories: (1) the liquid-feeding phase, when the vast majority of nutrients come from the ingested milk or milk replacer (MR), (2) the transition phase, during which the calf relies on both milk or MR and starter grain, and (3) the ruminant phase, at which time the calf can meet their nutritional needs solely from solid feed by way of the rumen (Davis and Clark, 1981; NRC, 2001). During the liquid-feeding phase, the omasum and abomasum are the main stomach compartments participating in the digestive process. Due to this, milk fed to the young calf will bypass the reticulum and rumen by way of closure of the reticular (esophageal) groove. As time goes on, an ever increasing microbial population colonizes the reticulum and rumen. Dry feed consumption during the transition phase allows for carbohydrate fermentation in the reticulo-rumen by the microbes, which yields volatile fatty acids (Davis and Drackley, 1998; Heinrichs et al., 2005). Acetic and propionic acids will be utilized by various tissues of the calf to meet its energy requirements; whereas, butyric acid will be used extensively by the epithelial cells of the rumen for papillae development, growth of rumen mass, and tissue metabolism (Baldwin et al., 2004). During all three calf development phases, water is the most crucial nutrient and cannot be neglected (NRC, 2001). The NRC (2001) guidelines for when to switch calves to a solely grain-based diet are based on feed intake rather than a particular day of the animal's life. These guidelines state that a calf is ready to be weaned once they have voluntarily consumed more than 0.68 kg/d of properly formulated starter grain for 3 d in a row.

In addition to undergoing many physical changes throughout the body during the first weeks of life, the calf's immune system also is developing in order to have the ability to mount a

proper immunological defense against any internal or environmental stressors beyond the protection provided by IgG obtained from its dam. A study by Husband et al. (1972) demonstrated that the calf doesn't produce significant amounts of immunoglobulins until approximately the second week of life. Investigation into the links among proper colostral IgG intake, immunological development, and early morbidity, and mortality of calves has now been underway for about 100 yr (Smith and Little, 1922). McEwan et al. (1970) were among the first to study Ig concentrations in calf serum and found that low serum Ig concentrations were more likely in calves that died due to diarrhea and septicemia. While the field is much better developed now, we will end this discussion by stressing again the importance of proper colostral IgG intake for lessening the risk of morbidity and mortality in the early weeks of the calf's life (Quigley et al., 2002).

Antioxidants and Vitamins

Antioxidant Overview

Antioxidants are substances that are capable of disposing of excess reactive molecules known as free radicals or reactive oxygen species (ROS). The ROS are important oxygen-containing molecules that are created during many metabolic processes. The main beneficial function of ROS is to maintain homeostasis by way of initiation of lipid peroxidation, cell signaling, host defense, and apoptosis. While these tasks are crucial to managing physiological processes, over-production of ROS can lead to the unfavorable breakdown of cell membranes, proteins, and nucleotides (Machlin and Bendich, 1987). As mentioned above, antioxidants can help reduce the amount of free radicals in the body. Balancing the necessary amount of ROS is a constant struggle that can be worsened during times of environmental, immunological, or metabolic stress (Albera and Kankofer, 2010).

This struggle is worsened during the transition period. Bernabucci et al. (2005) found that around the time of parturition, particularly immediately after, antioxidative status in the cow is decreased due to the high demand for assistance against ROS produced as a consequence of stressors. This drain of antioxidants allows for oxidative stress to surge, which in turn increases the risk for metabolic disorders. Cows that have a higher body condition score (BCS) coming into the transition period may have greater ROS production and thus may be especially sensitive to the spike in oxidative stress (Bernabucci et al., 2005). Combating this increase in oxidative stress by supplementing antioxidants during the transition period may be one way to assist the cow in meeting the three goals laid out by Goff and Horst (1997), as discussed earlier in the transition period section.

Calves, as with any living organism, can also experience an imbalance of oxidants and antioxidants. While they should not be fighting the same serious stressors as their dams, the first few weeks of life can be a difficult time for the calf while they undergo major changes to their physiological system and still-developing immunological system. If a calf also experiences external stressors, such as vaccinations or de-horning, or internal stressors, such as infections or nutrient shortage, during this time, significant oxidative stress may occur as a consequence. Some limited evidence shows that calves experiencing pneumonia are put under oxidative stress due to excessive phagocytic products such as $NO^{\cdot}$ and $O_2^{-\cdot}$ (Lykkesfeldt and Svendsen, 2007). Other studies show that thiobarbituric acid reactive substances (TBARS), lipid peroxides (LPO), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) may all be reliable disease markers in calves (Al-Qudah, 2009; Chigerwe, 2013). Lykkesfeldt and Svendesen (2007) concluded their excellent review of oxidative stress in farm animals by saying that while it is clear that antioxidants need to be more seriously considered as a treatment or preventative

measure for many diseases, it is difficult to increase our knowledge of the subject because "almost all of the large intervention studies using hard endpoints, such as mortality and morbidity, have been unsuccessful in linking antioxidant supplementation with lower disease risk or death."

There are many important enzymatic antioxidants, such as superoxide dismutase, glutathione peroxidase, and catalase, but the non-enzymatic antioxidants, which are α-tocopherol, retinol, β-carotene, and ascorbic acid, are the main focus of this paper. Many other compounds with antioxidant activity, such as tannins and flavones, exist in nature but are not nearly as potent so will not be included in the scope of this paper (Liu et al., 2013; Maciej et al., 2015; Jiang et al., 2016). Ascorbic acid, or vitamin C, is generally adequately synthesized in the bovine liver so its supply is rarely a concern for cattle producers (Matsui, 2012); consequently, it will not be discussed here in further detail. However, the lipid-soluble α-tocopherol (vitamin E), retinol (vitamin A), and β-carotene are commonly lacking in cattle and are thus of great interest to the dairy industry.

Vitamin E

Of the eight stereoisomers of vitamin E, *RRR*-α-tocopherol has the highest biopotency at 1.49 IU/mg and is the only form that occurs naturally (Combs, 2008). Although many synthetic forms are possible, various vitamin E esters, such as *RRR*-α-tocopherol acetate, are the type commonly used in animal feeding because they have high stability. Vitamin E within the body possibly plays roles in enzymatic function, signal transduction, and gene expression, but its fundamental function is to protect against oxidants (Combs, 2008). Vitamin E acts as a powerful antioxidant in two main ways. First, it supports the immune system by protecting neutrophils from being damaged by ROS during the intracellular killing of bacteria following phagocytosis.

Second, vitamin E acts as a scavenger of free radicals. It is also important to note that vitamin E can have a sparing effect on selenium, meaning that sufficient availability of vitamin E can allow selenium to better act in its own antioxidative capacity as a component of glutathione peroxidase (Weiss et al., 1983). Overall, the concentrations of vitamin E in blood serum, measured in conjunction with other lipid concentrations, can be a good marker of the amount of oxidative stress in the body (Miwa, 2011).

In dairy cows, higher blood vitamin E concentrations have been shown to lead to an increased neutrophil response (Hogan et al., 1992), decreased risk of retained placenta (LeBlanc et al., 2004), decreased incidence of stillbirth (Persson Waller et al., 2007), and, possibly, to decreased incidence of mastitis (Politis, 2012). Low blood vitamin E concentrations also may be an early indicator for occurrence of displaced abomasum (Qu et al., 2013). Also, Krueger et al. (2014) showed that vitamin E supplementation of 500 IU/d, which is well beyond the requirement (NRC, 2001), to pre-ruminant dairy calves tended to increase growth and possibly improve the health of the calves. Additional evidence indicates that supplementing vitamin E to calves enhances the immune response and decreases cortisol concentrations (Reddy et al., 1986; Reddy et al., 1987). Note that a deficiency of vitamin E in calves can cause skeletal myopathy, known as white muscle disease, or cardiomyopathy. However, the myopathy is more likely to be caused by selenium deficiency than a shortage of vitamin E so it important that both are supplied in sufficient amounts.

While fresh forages have high concentrations of α-tocopherol, the type of forage, field location, storage method, and length of storage all lead to great variation in the vitamin E content at time of feeding (Lindqvist et al., 2011) because *RRR*-α-tocopherol can be destroyed when exposed to oxygen or light (Combs, 2008). Because of this, the NRC (2001) established a

requirement of 0.8 IU/kg for lactating cows and suggested that vitamin E should be supplemented at a level of about 1,200 IU/d during the dry period and 545 IU/d during lactation. These recommendations can be reduced or perhaps even ignored if regular grazing occurs. The NRC (2001) set the required supplemental vitamin E for calves at 50 IU/kg of DM. Much of the research done since 2001 suggests the amount of supplemental vitamin E should be higher for cows than the current NRC recommended amounts. The requirement for calves is still debatable with more definitive work needing to be done. As there is low risk of vitamin E toxicity, over-supplementing is unequivocally better than under-supplementing for both cows and calves; however, vitamin E is one of the most expensive nutrients to supplement.

Vitamin A

While vitamin E is clearly an important antioxidant, vitamin A is arguably more influential overall as it affects vision, skin maintenance, skeletal metabolism, reproduction, epithelial cell differentiation, embryonic development, gene transcription, growth, and immune function, in addition to its own antioxidative activity. The exact antioxidative role of retinol is still unknown but studies by Livrea et al. (1995) and Baskin et al. (2000) indicated that vitamin A can protect low-density lipoproteins (LDL) from oxidation. It may also help lessen inflammation linked to oxidative stress caused by obesity (Puchau, 2011).

Vitamin A can be found as several vitamers. Retinol, the main form, is easily converted to structurally similar retinoids for storage or immediate use. Vitamin A is ingested as either retinyl esters from animal sources or as provitamin A carotenoids from animal or plant sources. The conversion of carotenoids to vitamin A is discussed in more detail in a later section. Dietary retinyl esters can be hydrolyzed to retinol in the small intestine. The fat-soluble retinol and retinyl esters are absorbed in the intestinal mucosa, incorporated into chylomicrons, and

transported to the liver by chylomicron remnants, then transported throughout the body by specific binding proteins (Combs, 2008). An insufficient amount of lipids in the diet can lead to decreased absorption of vitamin A, along with the other fat-soluble vitamins. Once absorbed, retinol can be stored in the retinyl ester form by way of re-esterification in the liver or metabolized to the active forms of retinal or retinoic acid. Vitamin A is excreted in either the feces or the urine, depending on the form.

As shown in Figure 1.1, retinol can be reversibly converted to retinal, which is the form of vitamin A that is crucial for proper vision. The vitamin A metabolite, 11-*cis*-retinal, in the presence of an opsin, allows for the detection of light. Vitamin A deficiency thus leads to compromised vision, especially in low light environments.

Retinal is converted to retinoic acid by way of an irreversible reaction. Retinoic acid is the form of vitamin A that plays a part in growth, development, and reproduction. Retinoic acid will bind to the retinoic acid receptors (RAR) or retinoid X receptors (RXR), which are often bound together as a heterodimer. The binding of retinoic acid to RAR or RXR will activate or deactivate various transcription factors (Marill et al., 2003).

While the actual amounts can vary between herds, studies in dairy cows and calves have identified the natural patterns of vitamin A found in transition cows, colostrum, and calves. The amount of vitamin A in cow plasma decreases markedly in the days leading up to parturition and increases again fairly soon following calving (Sutton et al., 1945; Goff and Stabel, 1990; LeBlanc et al., 2004). Goff and Stabel (1990) found the lowest levels of retinol at 1 d postpartum, with a 38% decrease compared to prepartal baseline concentrations. An earlier trial showed that vitamin A was lowest 3 d post-partum, with the rapid decline of plasma concentrations beginning 3 wk prepartum and resulting in an average decrease of 52% before

concentrations began rising (Sutton et al., 1945). Other research confirms that the vitamin A in cow plasma increases rapidly following parturition, with the concentration already significantly higher at 24 h post-partum when compared to the concentration immediately following parturition (Kankofer and Albera, 2008). In summary, while there is varying information on precisely when the nadir of plasma retinol occurs cows, it seems clear to be within the first few days following parturition.

Research as far back as 1933 demonstrated that colostrum, especially during the first 12 h postpartum, is much richer in vitamin A than is milk (Dann, 1933). A study comparing the vitamin changes at parturition between mastectomized and control cows found a much more pronounced drop in plasma retinol at parturition in the intact cows, indicating that colostrum production is responsible for much of the blood retinol decrease (Goff et al., 2002). Additionally, Bouda et al. (1980) showed that first colostrum contains about 10 times more vitamin A than the concentration found in plasma. These data all indicate that the decrease in blood vitamin A in cows around parturition is due to the partitioning of this crucial nutrient toward colostrum. Kankofer and Albera (2008) found that colostral vitamin A concentrations, as well as maternal plasma concentration, were higher at 24 h postpartum compared to immediately following parturition. A separate study also observed the highest retinol concentations in colostrum at approximately 24 h postpartum (Zanker et al., 2000). As the cow switches from colostrum production to normal milk secretion, the concentration of vitamin A in the milk decreases (Sutton et al., 1945; Parrish et al., 1949). It should be noted that primiparous cows secrete higher amounts of vitamin A in their colostrum than do multiparous cows, although the exact reason for this has not been identified (Parrish et al., 1949; Kume and Tanabe, 1993).

Many studies have shown that before receiving colostrum, blood vitamin A concentrations in calves are low, meaning placental transfer is limited and that colostrum is the crucial source of retinol for the neonate (Dann, 1932; Kume and Tanabe, 1993; Kume and Toharmat, 2001). In the 46 calves studied by Kume and Toharmat (2001), plasma vitamin A averaged 59 ng/mL immediately after birth. In the Bouda et al. (1980) study, plasma vitamin A of calves averaged 80 ng/mL prior to colostrum and almost doubled after colostrum intake (155 ng/mL) at 1 d of age. However, another study that sampled calf blood at 0 and 24 h of age showed no significant change in plasma retinol concentrations of calves during that relatively short time period (Kankofer and Albera, 2008). When comparing calf serum from d 1 and 6 of age, Kume and Tanabe (1993) found that the concentrations of vitamin A significantly increased, with this increase being corroborated by the similar research of Kume and Toharmat (2001). It had been shown previously that 75% of calves fed restricted amounts of vitamin A by way of colostrum became ill (Dvorak, 1960). Bouda et al. (1980), referring to the Dvorak research, postulated that improper epithelization due to hypovitaminosis A puts the calf at greater risk for alimentary and respiratory disease. Something Kankofer and Albera (2008) found that is of great interest was a positive correlation between the concentrations of retinol in cow plasma right after parturition and in calf plasma at 24 h of age, indicating that the vitamin A status of the dam affects retinol concentrations of the calf. It is still unclear how exactly the cow is effecting this change in the calf, but it is most likely largely driven by the passage of retinol through the colostrum. This finding again supports the pervasive research emphasizing proper colostrum intake by the neonatal calf. Overall, it is clear that vitamin A concentrations in calves are quite low prior to receiving colostrum, suggesting that placental transfer is limited; then calf vitamin A increases following consumption of colostrum and, while it may plateau until forage

consumption begins, continues to increase in the following days. More research is needed to understand the factors that contribute to the amount of vitamin A transferred in utero.

Accompanying the research to understand the natural vitamin A status in bovines, much work has been done to support the practical effects of vitamin A supplementation. Kankofer et al. (2010) showed that the total antioxidant activity and vitamin A concentration during the transition period was lower in cows with retained placental membranes. Along similar lines, an increase in vitamin A concentration prepartum was associated with a decreased risk of clinical mastitis during early lactation (LeBlanc et al., 2004). Spielman et al. (1947) showed that supplementing vitamin A in the ration during the dry period significantly increased the vitamin A content of colostrum. In this research study, supplemental vitamin A was provided as carotene, vitamin A ester form, or vitamin A alcohol form and, regardless of supplement type, the vitamin A found in colostrum was in the stable ester form. Research done in Japan again indicated that vitamin A supplementation to cows prepartum increased the concentration of retinol in the colostrum but did not affect plasma retinol concentrations or growth of the calves, even though the calves received dam-specific colostrum (Kumagai et al., 2001).

Supplementing retinyl palmitate, the primary retinyl ester, to calves by way of milk replacer resulted in a decrease in the amount of CD2, CD4, and CD8 cell antigens and interleukin-2 receptors expressed by leukocytes (Nonnecke et al., 1999). While these data demonstrate that vitamin A affects the immune system, there is much left to be done to understand the various factors involved before any further conclusions can be drawn about the immunological consequences of vitamin A supplementation. However, there is a large amount of evidence showing that supplemental vitamin A affects intestinal function and fecal consistency. Eicher et al. (1994) showed that calves supplemented with a high amount of retinyl

palmitate (87,000 IU/kg of milk replacer) had better fecal consistency and that calves that received both high amounts of vitamin A (87,000 IU/kg) and vitamin E (57 IU/kg of MR) had upregulated neutrophil activity. Another study also found that calves fed diets with a high amount of vitamin A, this time in the form of retinyl acetate, had less watery feces (Swanson et al., 2000). In contrast, a study in which calves were supplemented with 0, 15,000, or 30,000 IU/d of retinyl acetate found no difference in fecal scores, but there was an increase in treatment days when scouring calves were given an additional 30,000 IU/d of vitamin A (Franklin et al., 1998). Further studies should be done to compare retinoid sources when supplementing calves, along with how exactly vitamin A affects the digestive tract.

Eaton et al. (1970) suggested that calves are vitamin A deficient when plasma retinol concentrations are below 200 ng/mL, but Nonnecke et al. (1999) found that hepatic vitamin A can be sufficient even when plasma concentrations are less than 200 ng/mL, implying that low plasma concentrations might not consistently denote a systemic deficiency. A wide range of blood vitamin A concentrations have been associated with deficiency, many at levels far below those found by Eaton et al. (1970), which supports that just using blood retinol concentrations is not the most accurate way to judge the vitamin A status in calves (Swanson et al., 2000). Although the liver is the main storage location for retinol, Nonnecke et al. (1999) pointed out that blood samples are still predominantly used for vitamin A measurement due to the impracticality of taking liver samples on farms.

The requirements for supplemental vitamin E, vitamin A, and β-carotene are shown in Table 1.1. The 2001 NRC stipulated that supplemental vitamin A should be fed at a rate of 110 IU/kg of BW. The example rations given in the nutrient requirement tables of the NRC (2001) are formulated for over 80,000 IU/d for dry cows and 75,000 IU/d for lactating cows. For

calves, 110 IU/kg of BW translates to required concentrations of 9,000 IU/kg of DM and 4,000 IU/kg of DM for milk replacer and starter grain, respectively. Research conducted at the University of Illinois in which retinyl acetate was supplemented in milk replacer suggested that the requirement for vitamin A should be increased to 11,000 IU of vitamin A/kg of milk replacer DM (Swanson et al., 2000). Note that the concern over calf vitamin A consumption lessens once the calf begins ingesting forages, but some supplementation is always necessary for proper growth and development.

Similarly to vitamin E, unesterified vitamin A is present in forages but is sensitive to oxygen, light, and heat so will disappear rapidly following harvest (Combs, 2008). Unlike vitamin E, excess active vitamin A can be toxic so care should be taken not to over-supplement. Hypervitaminosis A causes many abnormalities, including bone softening, vision problems, muscular weakness, and skin peeling. While McDowell (1989) stressed the ill-effects of over supplementation, he stated that high levels of vitamin A in diets must be consumed by the animal regularly to see these negative effects, thus offering reassurance that short periods of accidental over-inclusion in the diet should not cause harm. The NRC (2001) suggested that the safe upper limit for cows is 66,000 IU/kg of DM, which is quite high relative to recommended feeding amounts. This limit will of course be lower in calves, but is not well elucidated at this time.

It is also interesting to note that various studies have suggested an interrelationship between vitamins A and E by showing that extremely high amounts of vitamin A may reduce vitamin E bioavailability (Dicks et al., 1959; Schelling et al., 1995; Zinn et al., 1996; Franklin et al., 1998; Nonnecke et al., 1999). However, the studies done by Eicher et al. (1994) and Swanson et al. (2000) found no effect of vitamin A supplementation on plasma vitamin E concentrations in young calves. Conversely, differing amounts of α-tocopherol may either increase or decrease

vitamin A concentrations, supporting an interrelationship between the vitamins but with an unidentified ideal combination of the two (Dicks et al., 1959). Franklin et al. (1998) suggested the importance of accounting for the vitamin A to vitamin E ratio in calves' diets. Unfortunately, the following discussion of carotenoids, specifically β-carotene, will only further convolute our understanding of the relationships between the fat-soluble vitamins.

β-Carotene

Carotenoids are fat-soluble pigments synthesized by plants as well as some photosynthetic bacteria and fungi. They can be stored in the adipose tissue or corpus luteum of the ovary, which is what gives these tissues their characteristic yellow tinge. Carotenoids are split into the two main classes of xanthophylls and carotenes, with carotenes being oxygen-free hydrocarbons that contain red and orange pigments. Some carotenes, such as β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin, are further grouped together by their β-ionone rings, which they have in common with retinoids. These four carotenoids can act either as a provitamin A or an antioxidant, with β-carotene being the most potent of the four. As shown in Figure 1.2, animals and humans can utilize the enzyme β-carotene 15,15'-dioxygenase, which is sometimes incorrectly called β-carotene 15,15'-monooxygenase, and an oxygen molecule to symmetrically cleave β-carotene into two all-*trans*-retinal molecules (ExPASy, 2016). β-Carotene 15,15'-dioxygenase is found mainly in the intestinal mucosa, but also in the liver and in the corpus luteum (Combs, 2008). The enzyme requires iron as a cofactor to make the conversion to retinal proceed and is enhanced by the presence of lipids. As discussed previously, retinal can then go on to either retinoic acid or retinol. The conversion of β-carotene to retinol will only proceed if there is a physiological need for vitamin A, which means that supplementing β-carotene is a way to supply ample vitamin A without causing hypervitaminosis A. It is

interesting, but not surprising, to note that β-carotene 15,15'-dioxygenase is found in the highest activities in herbivores, moderate activities in omnivores, and is completely absent in carnivores. This of course is due to their diet; carnivores consume very little of carotenoid-rich plants and thus meet all of their vitamin A and β-carotene needs by consuming animals that do have the ability to free vitamin A and carotenes from plants. Carotenoids that are not converted to retinal in the intestines are transported to the liver by way of chylomicrons, then re-packaged into lipoproteins. Due to its nonpolar nature, β-carotene is mostly transported by LDL.

While its provitamin A role is undeniably important, β-carotene itself can alternately act as a significant antioxidant. β-Carotene's many conjugated double bonds enable it to neutralize the unpaired electron of free radicals and can also reduce free radicals in low oxygen environments (Burton and Ingold, 1984; Combs, 2008). Although not as powerful an antioxidant as α-tocopherol, it has more antioxidative ability than retinol. Chew (1993), whose lab has done extensive work on β-carotene over the years, listed many immunomodulatory duties of carotenoids, with the main activity being to enhance lymphocyte action. In the same paper, Chew (1993) went on to point out that, by way of stimulating the immune system, β-carotene could improve the health and well-being of dairy cattle.

As mentioned before, interactions have been observed among vitamin E, vitamin A, and β-carotene. One example of this interaction is that β-carotene can act to protect tocopherols, allowing them to better carry out their antioxidative duties. Although the interactions among these three fat-soluble substances are beneficial to the animal, they make elucidation of the various pathways difficult. However, we do know through multiple studies that the natural serum concentration of β-carotene follows the same pattern as vitamin A; that is, β-carotene decreases in cows as they approach parturition and increases after calving, whereas calves are

born with little to no β-carotene in plasma but increase gradually after colostrum intake (Zanker et al., 2000; Kankofer and Albera, 2008).

This similar pattern probably contributed to the historical assumption that β-carotene was simply a means to the endproduct of vitamin A. However, Spielman et al. (1947) were possibly the first to stumble upon its other functions when they supplemented 1,000,000 IU/d of β-carotene to dry cows and saw no increase of vitamin A concentration in the colostrum. The research into β-carotene's non-provitamin A roles spiked in 1978, when Lotthammer, Cooke, and Friesecke all presented research regarding β-carotene's effect on the reproductive system. A literature review by Hemken and Bremel (1982) stated that the improved fertility observed from supplemental β-carotene when vitamin A status was adequate indicated that more work should be done on the topic. Bindas et al. (1984) followed this work up with an often-cited study that concluded β-carotene did not offer much benefit to the animal. Even still, much research followed. Although some trials, such as a recent study by Oliveira et al. (2015), showed little or no effect, in general studies showed that increased amounts of β-carotene in the corpus luteum and serum were associated with improved fertility in dairy cattle. An example of this convoluted research is shown in a study done in France by Kaewlamun et al. (2011). Cows in this trial were supplemented with 1,000 mg/d of β-carotene during the dry period. While the concentration of β-carotene in the colostrum and plasma was significantly increased by supplementation, there was no effect on the reproductive parameters of ovarian activity, production of progesterone, and diameter of the cervix and uterine horns. However, Kaewlamun et al. (2011) did use some less conclusive results to postulate that β-carotene supplementation may have reduced the involution period and inflammation of the uterus. Additionally, De Bie et al. (2016) found that

supplemental β-carotene increased the concentrations of vitamin A and β-carotene in the follicular fluid and improved follicular development.

Following the initial groundwork laid by early β-carotene researchers, further ramifications of β-carotene supplementation were explored. As stated earlier, much of the work done by the Chew lab group focused on the immunological effects of β-carotene. Subsequently, they reported the effects of supplemental β-carotene on immune response and metabolic disorders and found that β-carotene improved lymphocyte and phagocyte function, as well as decreased the incidence of metritis and retained placentas (Michal et al., 1994). A few years earlier, they tried to differentiate whether the improved phagocytosis was caused by β-carotene, retinol, or retinoic acid. While β-carotene did improve phagocytosis during certain periods around parturition, neither retinol nor retinoic acid ever did (Daniel et al., 1991). Additionally, Chew et al. (1993) demonstrated that β-carotene is taken up by lymphocytes in the blood. They postulated that β-carotene might improve immune action by protecting the lymphocytes from oxidative damage. Because of its impact on the immune system, Sordillo (2016) included β-carotene on her list of dietary supplements for improved immunity. Relating all of these studies back to the findings of Kaewlamun et al. (2011), perhaps the improved reproductive performance seen in some of the research was actually due to cows being better equipped for stronger or earlier estrous cycles.

Other researchers investigated the effects of β-carotene on colostrum and milk, with Rakes et al. (1985) one of the first to do so. This research study, which fed supplemental β-carotene at a level of 300 mg/d for the first 100 d of lactation, showed that while milk progesterone, milk yield, and milk fat percentage were not affected, somatic cell count (SCC) was lower in supplemented cows. A different study, which also supplemented β-carotene during

lactation at a moderate rate of 425 mg/d, contradicted the work of Rakes et al. (1985) by showing no significant effect on SCC but a significant increase in milk fat percentage (Ondarza et al., 2009). The latter study connected back to the improved fertility premise by suggesting that supplementing long-term during lactation might affect pregnancy rate. Kaewlamun et al. (2011) focused more on improving the vitamin status and quality of colostrum. Their results were that supplementation of β-carotene affected the concentration of β-carotene in the colostrum, but did not affect colostrum yield or IgG concentration. There also were no effects of treatment observed in the calves.

Another tack taken by researchers was to look more directly at the response to β-carotene intake by calves. An experiment done in Japan showed that the amount of β-carotene in the colostrum directly affected the amount in the calves' plasma 6 d after birth (Kume and Toharmat, 2001). These authors also noticed that the concentrations of β-carotene in plasma in the calves were remarkably low preceding colostrum intake, suggesting little to no placental transfer. This finding confirms the now generally accepted idea that the main source of β-carotene for calves is colostrum. The same study showed that both vitamin A and β-carotene concentrations in plasma were positively correlated with fecal dry matter percentage (DM%) at 6 d of age, with calves showing diarrhea having lower plasma concentrations of β-carotene. Further work is needed to clarify the mechanism by which β-carotene affects fecal DM%. When focusing on calves, Iwanska et al. (1986) found that calves from dams supplemented with β-carotene had higher serum concentrations of β-carotene and vitamin A, both initially and through the first weeks of life. Direct supplementation of 100 mg/d of β-carotene to some of the calves in the study showed a significant increase of β-carotene in the blood. Iwanska et al. (1986) concluded that

either supplementation to the dams or directly to the calves could improve β-carotene status in calves.

All in all, there has been a great deal of research conducted on β-carotene, but the results are so varied that it is still hard to unequivocally say whether supplementing β-carotene to dairy cattle is worth the expense. This variation could be attributable to differences in the status of vitamin A and vitamin E, oxidative stress from a myriad of reasons, amount and quality of forages in the diet, amount and type of lipids fed in the diet, breed of cow, age of cow, and possibly other factors. These discrepancies make the topic of β-carotene both a frustrating and intriguing one. Two things can be stated for certain: (1) much more precise research needs to be done, and (2) supplementation recommendations and effects may vary from farm to farm.

Conclusion and Thesis Objectives

After perusing the literature, it was determined that more research was needed to integrate β-carotene effects among the cow, colostrum, and calf. There are still many unanswered questions to test in a commercial setting. How does supplementing the cows with β-carotene affect the colostrum? How does it affect the calf? How do increased concentrations of β-carotene in the colostrum affect other components? Do increased concentrations of β-carotene in cow plasma cause any changes to metabolic markers or health outcomes? Are there any substantial effects on the calves even if they aren't supplemented directly? In summary, the objective of this research trial was to determine the effects of prepartum β-carotene supplementation on the cow, her colostrum, and her calf when the cow is provided with adequate amounts of vitamins A and E in her diet.

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Tables and Figures

Table 1.1. Requirements for supplemental vitamin E, vitamin A, and β-carotene. Adapted from NRC (2001) and DSM (2011).

	Lactation[1]	Far-Off[1]	Close-Up[1]	Fresh[1]	Calf[2]
Vitamin E – NRC (IU/d)	545[3]	1168[3]	1211[3]	545[3]	22
Vitamin E – DSM (IU/d)	555-1111	1111-3333	1111-3333	1111-3333	111-167
Vitamin A – NRC (IU/d)	69850	69850	69850	69850	4950
Vitamin A – DSM (IU/d)	100,000-150,000	75,000-100,000	75,000-100,000	75,000-100,000	20,000-30,000
β-Carotene – NRC (mg/d)	-	-	-	-	-
β-Carotene – DSM (mg/d)	300-500	500-1000	500-1000	500-800	100

[1] Assumed bodyweight of 635 kg.

[2] Assumed bodyweight of 45 kg. Assumed daily DM consumption of 431 g.

[3] Values taken from sample diets, pages 266-271. Listed value on page 168 of NRC is 0.8 IU/kg of BW for lactating cows.

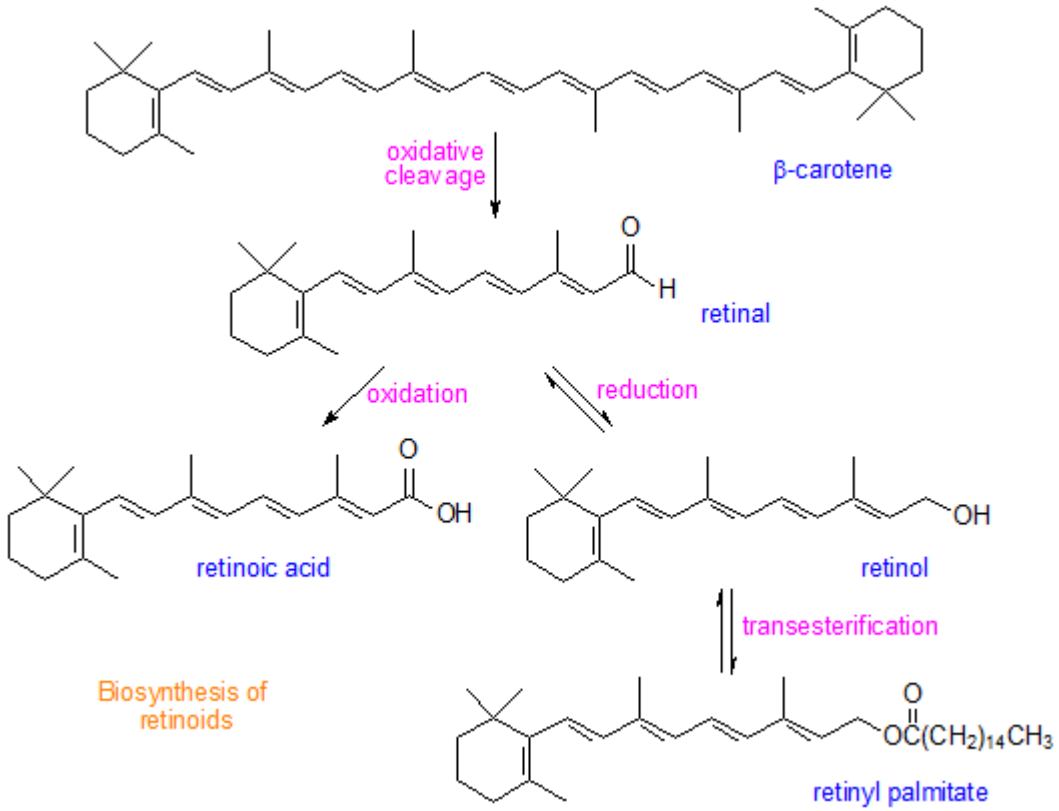

Figure 1.1. Vitamin A pathways. Adapted from William W. Christie, The Lipid Home, 2016. Accessed April 11, 2016.

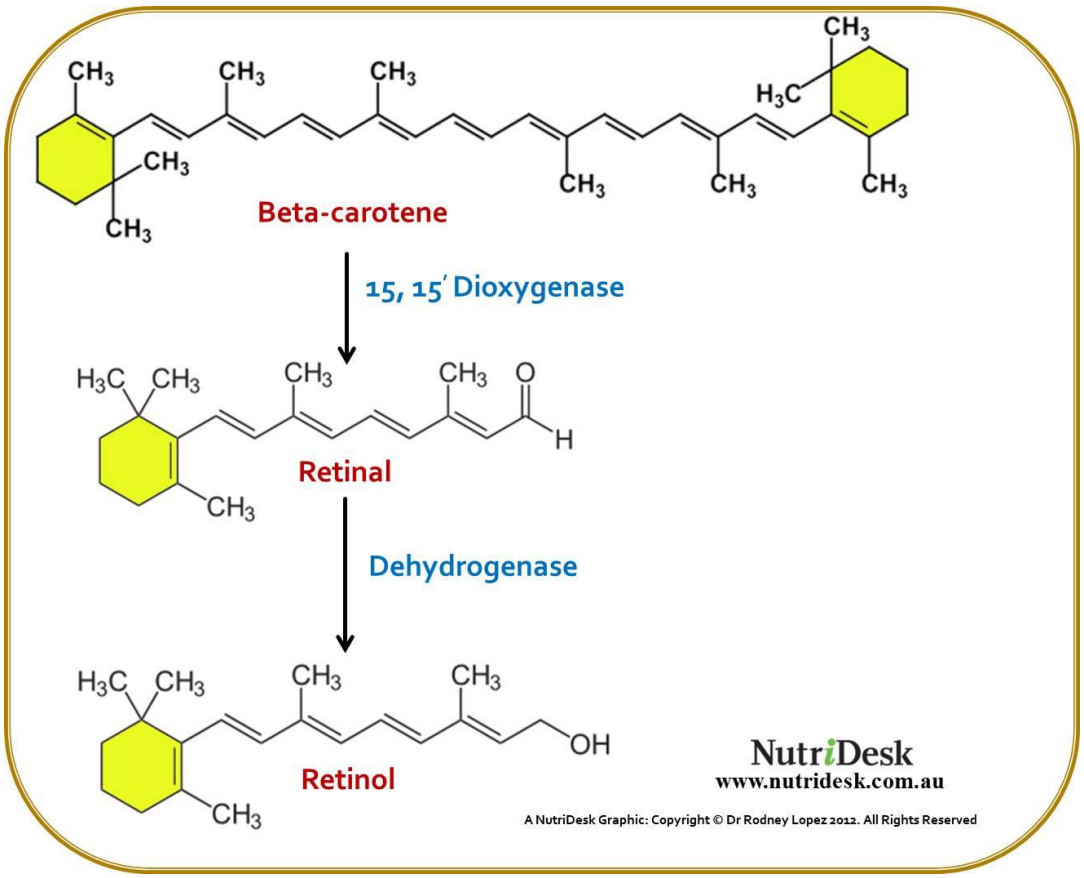

Figure 1.2. β-Carotene conversion to vitamin A. Adapted from NutriDesk, 2016. Accessed April 26, 2016. http://genomics.unl.edu/RBC_EDU/IMAGES/car2.jpg.

CHAPTER II: THE EFFECTS OF SUPPLEMENTATION OF β-CAROTENE DURING THE CLOSE-UP PERIOD ON COWS, COLOSTRUM, AND CALVES

Introduction

The investigation of alternate ways to improve animal health is of great importance for the welfare of the animal, profitability of the dairy operation, and satisfying the intensifying consumer demands. In order to decrease the amount of antibiotics and other drugs given to dairy cattle without diminishing profitability, dietary additives need to be explored and scrutinized more than ever. As the diets of dairy cattle get more and more fine-tuned, additives might not make the same impact as newly explored supplements did 30 yr ago; however, every bit of positive improvement that can be made to the diets may help us meet the end goal of improving cow health while keeping both the producer and consumer happy.

Some additives that show great potential for improving cow health are antioxidants, a class of molecules that help keep potentially harmful oxidative species in check. While oxidative species are necessary to help defend against invading pathogens and dispose of unnecessary, and potentially harmful, compounds in the body, an excess of reactive oxidative species (ROS) can damage necessary cellular structures (Machlin and Bendich, 1987, Albera and Kankofer, 2009). If antioxidants are limiting, ROS can cause oxidative stress to the animal leading to possible breakdown of crucial biological tissues. Around the time of parturition, metabolic activity in both the cow and calf increases, causing a surge of ROS and thus oxidative stress (Castillo et al., 2005, Albera and Kankofer, 2010). Any additional environmental, metabolic, or immunological stressors can further increase the amount of ROS and potentially overwhelm the antioxidant system.

Over the past 20 yr the transition period has been elucidated as a critical period for a dairy cow. The steps undertaken by the cow in an attempt to correct her energy balance can lead

to negative consequences, such as the onset of metabolic diseases, mastitis, and other health issues (Goff and Horst, 1997). These metabolic changes tack on additional oxidative stress to the animal, which then weakens tissues by breaking down cellular membranes and then puts the animal more at risk for immunological and metabolic issues (Bernabucci et al., 2005). This vicious cycle can either be chased by managing symptoms or it can be addressed more directly by way of antioxidants.

In a similar manner, calves undergo many changes in the first weeks of life that can cause their ROS load to spike. Namely, their physiological system is changing from that of a pre-ruminant to one of a ruminant and their fairly naive immune system is struggling to become competent (Lykkesfeldt and Svendsen, 2007). While a fair amount of research with antioxidant use in calves has been done to this point, they are still perhaps underutilized by calf raisers.

Three lipid-soluble, non-enzymatic antioxidants will be investigated during this research trial: vitamin E, vitamin A, and β-carotene. Vitamin E has long been known as a powerful antioxidant, with much research demonstrating its efficacy. Similarly, vitamin A is recognized as a critical nutrient in the diet of the dairy cow. However, vitamin A primarily carries out non-antioxidative functions, such as epithelial maintenance and gene expression, and is not nearly as robust of an antioxidant as vitamin E (Combs, 2008). Finally, β-carotene is a dual-purpose nutrient, either acting as provitamin A or directly as an antioxidant. β-Carotene has long been recognized for its ability to be split into two retinol molecules and has long been supplemented as a source of vitamin A. However, research done in the 1970's began to reveal how impactful β-carotene can be in its own right. While the exact mechanisms remain unclear, β-carotene possesses immunomodulatory capabilities, possibly by way of scavenging singlet oxygen-suppressing peroxyl radicals (Chew, 1993). It is important to note that β-carotene is only

converted to vitamin A as needed, so meeting the vitamin A needs of an animal means that any supplemented β-carotene should be able to be utilized as an antioxidant.

Although significant research in the field of carotenoids has been conducted in the past few decades, the exact mechanisms of how and when β-carotene's antioxidative activities occur and especially how β-carotene interacts with vitamins A and E still need further study. Much conflicting research exists regarding these interactions (Dicks et al., 1959; Eicher et al., 1994; Schelling et al., 1995; Zinn et al., 1996; Franklin et al., 1998; Nonnecke et al., 1999; Swanson et al., 2000), suggesting that there are unseen variables at play that must be identified and addressed. Also, many experiments have studied vitamin A, vitamin E, and β-carotene solely in cows or calves, but more are needed to examine the relationships among the three. Thus, the objective of this research trial was to identify the effects of prepartum supplementation of β-carotene to cows with adequate vitamin A and E status on the cow, her colostrum, and her calf.

Materials and Methods

Description of Commercial Farm

The research trial was conducted on a large commercial dairy farm in northern Indiana. The farm was selected for its large herd size, modern facilities, strict adherence to protocols, well-maintained computer records, and commitment to research. The facilities of particular interest included headlocks in the close-up pens, headlocks in the fresh pens, sanitary maternity area, headlock with stand-alone milking setup in maternity area, individual calf housing, ice maker, and an empty chest freezer. The dry cow freestall barns, which included the maternity area, and calf raising facilities were located on separate sites near the main dairy site. The calf ranch was located approximately halfway between the two. Upon freshening, cows were milked in the maternity area. Twice daily, newly freshened cows were taken by stock trailer to the fresh

cow pens at the main dairy site. Once daily, new calves were taken by stock trailer to the calf ranch.

The herd consisted of approximately 7,000 cows in milk and 675 dry cows. The herd had an average pregnancy rate of 18% and an average 305-d mature equivalent milk yield of 14,000 kg. Due to the size of the farm, two milking parlors were needed and the herd was thus split into two smaller herds. The second milking parlor and the corresponding freestall barns were built on the same site as the first in an identical but mirrored footprint. The two herds shared the feed storage, which was located between the two parlors, and the employees split their time between the two. The crews who fed the cows and did fresh checks each day were the same for each herd. In other words, the same employee would mix the ration for the cows of both herds, the same team of employees would work with the fresh cows from both herds, and the same team of employees would complete treatments and surgeries for both herds. For computer-record purposes, the two herds were treated as one, but could be sorted against each other using differing pen numbers. Despite the near-identical management and facilities, the herds themselves were treated as stand-alone herds with mixing of the cows between herds kept to a minimum. Different colored ear tags were used for each herd and the daughters were kept in the respective herd of their dam. Similarly, at the dry cow facilities, there were two identical sets of freestall barns, separated by the central manure lagoons and maternity area. The dry cows were fed the same rations from the same feedstuffs mixed as one batch in the same wagon by the same employee. The employees at the dry cow facility performed their tasks on both herds without prejudice. Immediately prior to freshening, cows from each herd would be channeled into the maternity area, where they could possibly be mixed from cows from the other herd. After freshening, cows from both herds were kept in the same pen and hauled on the same stock trailer

before being separated again at the milking site. Other than the maternity area, cows were rarely, if ever, exposed to cows from the other herd. At the calf ranch, calves from both of the herds, along with calves from a neighboring large dairy, were kept in individual hutches but had the possibility of being housed next to calves from a different herd.

Selection for Trial

Holstein cows were selected based on their expected calving date for an expected calving window of June 29 to July 12, 2015. This narrow calving window was dictated by the farm management as they had committed their animals to other research projects. Only cows that had completed at least one lactation were selected, meaning no first-calving heifers were used. The 139 multiparous cows with expected calving dates within the desired window were first blocked by parity, then balanced by previous lactation milk yield. They were then randomly assigned to either the treatment or control group. Ninety-four Holstein cows with a mean parity of 2.4 ± 0.63 (mean ± SD), 52.5 ± 4.45 d dry, and a calving date -0.8 ± 3.65 d from expected calving completed the full trial. Fifty-two of the cows were housed in one pen while the remaining 42 animals were from another.

Cows: Management, Treatments, and Sampling

All experimental procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. From June 8 to July 17, the cows in the close-up pens were locked in the headlocks (DaSilveira Southwest, Inc.; Madisonville, TX) each morning at feeding time. The cows were locked up for less than 2 h each day, with the goal being less than 1 h when possible.

Cows were enrolled on trial 21 d prior (d -21) to expected calving date. On the enrollment date, each cow was assigned a body condition score (BCS) according to the system

published by Ferguson et al. (1994) and had a small colored ear tag (Destron Fearing; Langeskov, Denmark), blue for supplementation and purple for control, attached to the bottom corner of their existing ear tag using an Universal Total Tagger (Allflex; St. Hyacinthe, Quebec, Canada). They were also given their first supplementation dose, either control or treatment, and blood samples were taken from the caudal vein into two 10-mL red/gray stopper evacuated serum separation tubes and one 10-mL lavender Hemogard closure evacuated K_2EDTA whole blood tube (Becton Dickinson; Rutherford, NJ). Following sample collection, the whole blood tubes were immediately placed on ice in a dark cooler. The serum tubes were placed in the deep pockets of dark-colored coveralls while other samples were being collected and wrapped in foil once removed from the pockets.

The control treatment (Con) consisted of 50 g of cracked corn (University of Illinois Feed Mill; Champaign, IL), 50 g of dry molasses flakes (Dri-Mol, Archer Daniels Midland; Chicago, IL), 50 g of shredded beet pulp (Midwest Agri-Commodities; San Rafael, CA), and 25 g of XPC yeast (Diamond V, Cedar Rapids, IA). The mixture for the β-carotene treatment (βC) was the same with the addition of 8 g of Rovimix β-Carotene (DSM; Heerlen, Netherlands), which is 10% β-carotene so contained 800 mg of β-carotene. The amount of βC was determined by reviewing previous research as well as manufacturer recommendations. The treatment mixtures were formulated to maximize palatability while still providing a good carrier blend for the β-carotene supplement. The mixtures were weighed out using a digital scale (Best Harvest; Largo, FL) into quart-sized plastic bags (Great Value; Bentonville, AR) that had differing color zip tops for the Con and βC treatments.

The treatments were fed as a topdress to the cows while they were locked in headlocks each day. The cows received the topdress from d -21 until calving. A daily worksheet was used

to track which cows received which treatment each day. The different colored ear tags and different colored bags also provided a quick reference as to which treatment they were to receive. Topdresses were placed on top of the TMR each morning within 2 h of the TMR being placed in front of the cows. The TMR was mixed and fed according to the farm's protocols. A small divot was made on top of the TMR pile and the contents of the appropriate bag were carefully expelled into the divot. Each cow was watched to ensure that they didn't mix the topdress into their food and that the neighboring cows did not consume any of the topdress. If the neighboring cows were not easily dissuaded from consuming the topdress, they were released from their headlock and the headlock was locked in the closed position so no cows could re-enter that space. The cows were graded each day on the amount of topdress consumed. They were scored as follows:

0 – did not consume any

1 – did not consume a substantial amount, but exhibited licking behavior

2 – consumed roughly half

3 – consumed almost all

4 – consumed completely

See Appendix A1 for a visual representation of each score.

If topdress consumption was scored ≤ 2 that cow was fed an additional dose of topdress the following day (i.e., two doses). The number of doses to be fed to each cow was indicated on the daily worksheet. Failure of the cow to consume most of the topdress (score ≥ 3) for 3 d consecutively resulted in the cow being removed from trial in order to ensure that cows used for analyses maintained serum β-carotene concentrations throughout the topdress period. Cows were given these three chances because βC is a fat-soluble substance that is stored by the body,

making it unnecessary to be consumed every day. According to research at the University of Florida (unpublished data), a single dose of β-carotene can cause an increase in the concentration of β-carotene in serum that persists for several days following consumption (C. Staples, personal communication, July 7, 2016). Of the 139 cows that began the experiment, 40 cows (23 from the βC group and 17 from the Con group) were removed from trial due to failure of sufficient topdress consumption. If cows consumed the treatment for fewer than 14 d, meaning they calved more than 7 d early, they were removed from the trial. They were also removed from trial if they calves more than 7 d late. Two cows (both from the βC group) were removed from trial for calving early and three (2 from Con group and 1 from βC group) were removed for calving late. Any cows removed from trial due to failure to consume topdress or calving more than one week from expected calving date had their treatment ear tags removed and had no more samples collected from them.

In addition to d -21, caudal blood draws, using two serum tubes and one whole blood tube as described above, were performed at 7 d (d -7) prior to calving, within 2 h of calving (d 0), and 7 d following calving (d 7). Except for d 0, blood draws could be taken ±1 day. The d 0 blood samples were taken within 2 h of calving while the cow was locked up in the individual headlock to be milked. The d -21 and d -7 blood draws were performed during the morning lock-up period. Similarly, d 7 blood samples were taken during the time period that the fresh cow management team locked the fresh cows up each morning. Following the d 7 blood draw, the cow was assigned a BCS and the treatment ear tag was removed using an EZ-Knife Ear Tag Remover (Destron Fearing; Langeskov, Denmark).

The cows were cared for by the farm staff according to their protocols. Cows calved on clean straw in a pen that was sanitized regularly and were milked within 2 h of calving. Disease

incidence, treatments, and reproductive status were entered into DairyComp 305 (Valley Agricultural Software; Tulare, CA) by the farm staff. Calving difficulty was determined by the research team after conferring with farm staff based on the system developed by the University of Minnesota (Minneapolis, MN). The scoring system was as follows:

1 – no problem (unobserved or less than 2 h)

2 – slight problem (greater than 2 h, but no assistance provided)

3 – needed assistance (hand pull)

4 – difficult pull (obstetrical chains with considerable force)

5 – extreme difficulty (mechanical puller or cesarean section)

Calves: Management and Sampling

All calves of the cows on trial were given 3.78 L of colostrum from their specific dam within 2 h of birth. Dam-specific colostrum was given regardless of colostrum quality. If the cow produced less than 0.95 L of colostrum, additional colostrum was fed from a cow assigned to the same treatment. This extra colostrum was stored in the refrigerator by the staff according to farm protocols and marked with the treatment and cow identification. All other care following calving, such as navel dipping and second colostrum feeding, was done by the employees per their protocols. Heifer calves received the same color ear tag as their dam while all bull calves received a different color tag.

Blood was sampled from the jugular vein within 2 h of birth (d 0), at 24 h ± 6 h (h 24) following birth, at 7 d of age (d 7), and again at 60 d of age (d 60). As with the cows, the samples were collected into two 10-mL red/gray stopper evacuated serum separation tubes and one 10-mL lavender Hemogard closure evacuated K_2EDTA whole blood tube. The whole blood

tubes were again placed on ice in a dark cooler and the serum tubes were placed in dark pockets or wrapped with aluminum foil.

Following the first colostrum feeding, the calves were fed and cared for according to the farm's protocols. Twice daily they were given pasteurized whole milk supplemented with milk replacer when needed, along with starter and water. No supplemental β-carotene was given to the calves. Calves were weaned at 56 d of age but not moved to group pens until approximately 70 d of age. The d 60 samples were meant to represent a time period where the calves were under heightened stress from weaning and their changing diet. Ideally, blood samples would be taken again after being moved to group housing, but the facilities did not make that practical for this study.

Once a week, the calves were assigned a health score by the research team according to the metrics developed by the University of Wisconsin (Madison, WI). The health scores included fecal, respiratory, nasal, ocular, and ear. As with the cows, treatment and other notable health incident data were retrieved from DairyComp 305 records.

Colostrum

Within 2 h of calving, each cow was milked and colostrum samples were placed into two 15-mL plastic conical Falcon tubes (Becton Dickinson; Rutherford, NJ) and two 60 mL flip-top milk sampling vials (Thermo Scientific; Waltham, MA). The remaining colostrum was then fed to the calf as described above. The colostrum was immediately assigned a color score on a 1 to 4 scale established for this study, with a score of one being almost white and four having the color of orange juice. This color grading system will be further developed by DSM.

Feed

Feed samples were obtained on a weekly basis. For the close-up cows, samples were gathered into 3.8 L-sized Ziploc bags (S.C. Johnson; Bay City, MI) from untouched TMR and refused TMR from both pens, corn silage, hay, and premix pellets. During the weeks in which trial cows were in the fresh pens, samples were similarly taken from their TMR and corn silage.

Sample Analysis and Storage

Within 2 h of blood collection, the two serum tubes were centrifuged with a HN-SII centrifuge (Damon IEC; Needham Heights, MA) at 1300 × *g* for 15 min. The serum was pipetted into 5-mL polystyrene tubes (Globe Scientific; Paramus, NJ) and stored at -20°C in the on-farm chest freezer. The whole blood was used for on-farm β-carotene analysis using iEx vials and an iCheck reader (BioAnalyt; Teltow, Germany), which was validated by Raila et al. (2012). The remaining whole blood was pipetted into 5-mL polystyrene tubes and stored at -20°C. Periodically throughout the study, the frozen samples were transported on ice by car back to the University of Illinois where they were again stored at -20°C. Serum samples were taken on ice to the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University (East Lansing, MI) for high performance liquid chromatography (HPLC) analysis of vitamin A, vitamin E, β-carotene, cholesterol, and vitamin E to cholesterol ratio, as well as to the University of Illinois College of Veterinary Medicine Diagnostic Laboratory (Urbana, IL) for automated enzymatic analysis of metabolites and enzymes. These metabolites included creatinine, blood urea nitrogen (BUN), total protein (TP), albumin, globulin, calcium (Ca), phosphorus (P), sodium (Na), potassium (K), chloride, glucose, total alkaline phosphatase, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), total bilirubin, creatine phosphokinase (CPK), total cholesterol, glutamate dehydrogenase (GLDH),

bicarbonate, magnesium (Mg), triglycerides, and anion gap. Assays for NEFA and BHBA (Wako Diagnostics; Mountain View, CA) were performed in-house.

The serum from the calves was centrifuged, stored, and analyzed identically to the cow serum with two notable additions. Following centrifugation, the serum was checked for total protein using a digital refractometer (Misco; Solon, OH). Second, the samples obtained at d 0 and h 24 were sent on ice to Prairie Diagnostic Services (Saskatoon, Saskatchewan, Canada) to be analyzed for IgG concentration. As with the cows, the whole blood from the calves was tested for βC content and stored at -20°C.

The colostrum in the two Falcon tubes was immediately frozen at -20°C and later transported to the on-campus freezer. One of the tubes was taken to DCPAH for vitamin analysis, then sent on ice to Prairie Diagnostic Services for IgG analysis, while the second tube remained in storage. The first flip-top vial of colostrum immediately had a Broad Spectrum Microtab II preservative tablet (D&F Control Systems, Inc.; San Ramon, CA) added and was then refrigerated at 4°C. Twice weekly, the vials with the preservative tablets were sent to Dairy Lab Services (Dubuque, IA) to be analyzed for fat, protein, somatic cell count, lactose, other solids, total solids, and milk urea nitrogen. The second flip-top vial was used for the color scoring as described above, βC analysis with the iCheck, Brix reading with the Misco refractometer, and then refrigerated at 4°C. No preservative tablet was added to these samples as it would alter the color of the colostrum. Also twice weekly, these vials were taken back to the University of Illinois where the color was analyzed using a colorimeter (Konica Minolta; Tokyo, Japan). Colorimetry is similar to spectrophotometry, but is modified to account for human perception of color. Colorimeter results were on the L*a*b* color space scale. L* measures lightness, a* measures red-green, and b* measures yellow-blue.

Following collection, the TMR samples were all processed through a Penn State Particle Separator and weighed according to the protocol published by Pennsylvania State University (State College, PA). All feed samples were then frozen at -20°C in the on-farm freezer and later transported back to the campus freezer. Samples were composited as shown in Table 2.5 without being dried. Composited samples were sent to DCPAH for vitamin analysis and to Dairy One (Ithaca, NY) for the wet chemistry model profile.

Statistical Analysis

Cow was considered to be the experimental unit since treatments were applied individually to cows during feeding. Cows from both treatments were housed in the same pen, and pen was replicated so that environmental effects could be adequately accounted for in the statistical models. As shown in Table 2.1, mean parity, mean previous lactation milk yield, and number of days dry were not different between the treatment groups.

The collected data were divided into cow serum, calf serum, colostrum, and cow health datasets with each then analyzed using models developed in SAS 9.4 (SAS Institute Inc., Cary, NC). Cow production and health parameters taken from trial records and from a DairyComp backup obtained approximately 9 mo after the beginning of the trial included parity, number of days dry, calving date, days open before being rebred, times bred, reproductive status, whether they left the herd, days in milk at time of the backup, predicted 305-day milk yield, milk yield at d 28 postpartum, day of peak yield, amount of peak yield, and change in BCS, as well as the presence or absence of displaced abomasum, off feed, ketosis, lameness, footrot, mastitis, metritis, milk fever, and retained placenta.

PROC UNIVARIATE was used on each combination of variable, time point, and treatment assignment to examine residual plots and Shapiro-Wilk test statistics for normality.

Outliers greater than 5 standard deviations from the median were removed. Following deletion of extreme outliers, the Brown-Forsythe test in PROC GLM was used to test each variable for equal variances. Transformations were not performed to obtain normality, but were performed where necessary to achieve homogenous variances. Log transformations were executed on GLDH from the cow serum dataset, GGT and bilirubin from calf serum data, b* from colostrum color data, and BCS from cow health data. Additionally, a square transformation was executed on GGT from the cow serum dataset. β-Carotene, fat percentage, and SCC were left untransformed for the colostrum data.

Continuous variables were used to construct mixed models for a completely randomized design in PROC MIXED and analysis of variance was examined for each variable. PROQ FREQ was also utilized for calf serum data. The parity and pen numbers of the cows were used as fixed effects. Parity was included in the model due to known milk yield and vitamin differences by parity. Of the 94 cows used for the trial, 61 were beginning their 2^{nd} lactation, 26 were beginning their 3^{rd} lactation, and 7 were beginning their 4^{th} lactation. The time point was included in the model for both cow and calf serum data and analyzed as a repeated measure. Data from date of enrollment (d -21) were used as a covariate for the cow serum data. A simple covariance structure was used for the cow data while an autoregressive(1) structure was used for the calf data. Various interactions were investigated for significance. Binary values from the cow health dataset were analyzed using PROC GLIMMIX with a value of 1 representing that they were pregnant, sick, or sold depending on the variable and a value of 0 representing the opposite. Reproductive values were analyzed at a cut-off point of 120 DIM, while health values were analyzed using data from ≤ 30 DIM. Correlations between variables were inspected using

PROC CORR. Statistical tests were deemed as significant when $P < 0.05$ and as trending towards significance when $0.05 < P < 0.10$.

Results

Diet Composition and Vitamin Content in the Feeds and Rations

Ingredient formulation and analyzed composition of the closeup cow diets are shown in Tables 2.2, 2.3, and 2.4. Results for the vitamins found in the tested feedstuffs are reported in Table 2.5. Note that results were variable, which was expected due to the natural disappearance of vitamin A, vitamin E, and β-carotene following harvest. Table 2.6 compares the actual values in the tested feed against the NRC (2001) requirements and DSM (2011) recommendations. Vitamin E values were 1,115 IU/d and 507 IU/d in the close-up and fresh rations, respectively. The values for vitamin A were determined to be 84,455 IU/d in the close-up ration and 263,156 IU/d in the fresh ration. Penn State particle separator results are shown in Tables A1 and A2.

Cow Serum

The results of the analyses on the cow serum are presented in Table 2.7. There was a treatment effect with no treatment by parity interaction for β-carotene ($P < 0.01$) and vitamin A ($P = 0.023$). Overall, β-carotene concentration of treated cows was 2.95 μg/mL compared to 0.71 μg/mL for control cows. There was a treatment by day interaction ($P < 0.01$), with the serum concentrations by treatment and day shown in Figure 2.1. At d -21, before receiving supplementation, the herd average was 0.85 μg/mL. This was quite low compared to the generally accepted serum β-carotene concentration of 3.0 μg/mL (Frye et al., 1991). By d -7, the mean concentration for the supplemented cows had increased to 3.45 μg/mL, with the peak of 3.64 μg/mL at d 0. In contrast, the concentrations in the non-supplemented cows never went

beyond 0.86 μg/mL, which was measured at d -7. The lowest β-carotene concentrations occurred at d 7, with values of 1.77 μg/mL and 0.56 μg/mL for βC and control cows respectively. The vitamin A concentrations by treatment and day can be seen in Figure 2.2. Prior to supplementation, the serum retinol average of the cows sampled was 247 ng/mL, which is at the low end of the adequate range outlined in the Merck Veterinary Manual (Kahn, 2010), and fell to its nadir at d 0 of 137 ng/mL for supplemented cows and 105 ng/mL for control cows. The dynamics of cow serum vitamin E by treatment and day are shown in Figure 2.3. Note that there was no effect of β-carotene on vitamin E. Vitamin E concentrations at d -21, d -7, and d 0 were within the ideal range of 2 to 4 μg/mL for both treatment groups; however, they were below that range at d 7. Overall, vitamin E was adequate and independent from the effects of β-carotene supplementation.

While there was no treatment by parity interaction for the vitamins, there was for many other serum variables. The variables that showed a treatment effect or tendency towards effect along with a treatment by parity interaction were albumin ($P < 0.029$), globulin ($P < 0.01$), and the ratio of the two ($P < 0.01$). There was an independent treatment effect or trend for TP ($P = 0.045$) and chloride ($P = 0.080$). The effect of day was significant for all variables except for GGT, CPK, and GLDH. Serum CPK had a significant treatment by day interaction ($P = 0.03$), but did not have an overall treatment effect as it was not significant for βC cows at -7 d and 0 d. The pen effect was negligible but could not be removed from the model due to a tendency for vitamin A ($P = 0.068$) and cholesterol ($P = 0.070$). There were no pen by treatment interactions. Many of the variables differed by parity, including vitamins A ($P < 0.01$) and E ($P < 0.01$), but β-carotene ($P = 0.94$) did not.

β-Carotene concentration had little to no correlations of note with other metabolites in cow serum. The strongest correlations were with vitamin E at 0.24 ($P < 0.01$), AST at -0.26 ($P < 0.01$), and bicarbonate at -0.25 ($P < 0.01$). β-Carotene had a weak correlation of -0.13 ($P < 0.033$) with BHBA, but no correlation with NEFA. Vitamins A and E were moderately correlated at 0.48 ($P < 0.01$). Vitamin A was moderately and negatively correlated with both NEFA and BHBA at -0.63 and -0.49 ($P < 0.01$ for each), respectively, while vitamin E had correlations of -0.42 and -0.49 ($P < 0.01$ for each).

Cow Health

The results for the continuous variables from the cow health data are shown in Table 2.8. No variables were affected by treatment. The only pen effect detected was a weak tendency for significance for days to conception ($P = 0.098$). There were no interactions of treatment and parity. Number of days dry prior to calving ($P < 0.01$) and milk yield at 30 DIM ($P = 0.026$) were the only variables affected by parity. Binomial variable results are presented in Table 2.9. No variables were found to have a significant effect of treatment.

Colostrum

The results for colostrum analysis are shown in Table 2.10. There was a treatment effect for β-carotene ($P < 0.01$), a* ($P = 0.014$), b* ($P < 0.01$), and fat percentage ($P = 0.042$). The concentration of β-carotene in the colostrum was 1.53 μg/mL for cows on the β-carotene treatment and 0.64 μg/mL for cows on the control treatment. Color score was significantly increased by β-carotene supplementation ($P < 0.01$), as shown in Table 2.11. The mean color score for βC cows was 3.23 while it was 2.40 for Con cows. A visual representation of each color score, along with the mean β-carotene concentration in the colostrum for each score, is

shown in Table 2.12. Note that no cows on the trial had a colostrum color score of 1. The mean β-carotene of colostrum scored 2 was 0.56 µg/mL, while for scores 3 and 4 the concentration increased to 0.93 µg/mL and 1.72 µg/mL, respectively.

The treatment and parity interaction was not significant for any variables, but did trend towards significance for fat percentage ($P = 0.085$) and lactose percentage ($P = 0.066$). The least squares (LS) means for fat percentage by treatment and parity are shown in Table 2.13. Note that for βC cows, fat percentage increased as parity did, with cows in their fourth lactation having a fat percentage of 5.34. However, fat percentage for Con cows did not show the same pattern; it reached its peak in cows in their second lactation and nadir in cows in their third lactation before rising again for the oldest cows. An effect by parity was seen for L* ($P < 0.01$), b* ($P = 0.022$), IgG ($P < 0.01$), BRIX ($P < 0.01$), and vitamin A ($P = 0.017$). There were some pen effects detected for the colostrum variables. The pen effect was significant for a* ($P = 0.044$), vitamin E ($P < 0.01$), and β-carotene ($P = 0.046$), and had a tendency for b* ($P = 0.079$). There were no treatment by pen interactions.

Correlations found between the vitamins and other variables in the colostrum are shown in Table 2.14. Colostral β-carotene had moderate positive correlations with vitamin E of 0.46 ($P < 0.01$), vitamin A of 0.42 ($P < 0.01$), a* of 0.48 ($P < 0.01$), b* of 0.54 ($P <0 .01$), and fat percentage of 0.44 ($P < 0.01$). In addition to β-carotene, vitamin E was moderately correlated to BRIX and fat percentage at 0.55 and 0.45, respectively ($P < 0.01$ for both). Vitamin E also had weaker correlations with vitamin A at 0.31 ($P < 0.01$), IgG at 0.37 ($P < 0.01$), and a* at 0.39 ($P < 0.01$). Vitamin A, in addition to its correlations with β-carotene and vitamin E, was weakly correlated with a* ($r = 0.32$, $P < 0.01$) and fat percentage ($r = 0.34$, $P < 0.01$).

Calf Serum

β-Carotene was not able to be analyzed with Proc Mixed for the calf serum data due to many of the observations having non-detectable concentrations.. Due to this, Proc Freq was utilized to investigate whether there were any treatment differences for the number of calves having concentrations above the detection threshold of 0.05 μg/mL. Results are shown in Figure 2.4. Treatment was significant for calves at 24 h ($P < 0.01$) and d 7 ($P = 0.047$). At 24 h, 89.3% of calves found to be above the β-carotene detection threshold were from β-carotene treated cows. Similarly, at d 7, 85.7% of calves above the threshold were from cows supplemented with β-carotene. At d 0 only one calf (from a Con cow) had detectable β-carotene concentrations and at d 60 no calves did. The maximal number of calves with β-carotene concentrations above the threshold occurred at d 7 (n = 28).

As shown in Table 2.15, the effect by time point was significant for all calf serum variables. There was a significant or trending treatment by time interaction for alkaline phosphatase ($P = 0.071$), GGT ($P < 0.01$), bicarbonate ($P = 0.057$), and triglycerides ($P = 0.011$). The only significant overall treatment effects were seen for BUN ($P = 0.041$) and GGT ($P < 0.01$); however, both are confounded by a trend for a treatment by parity interaction. The LS means by treatment and parity for BUN and GGT are in Tables 2.16 and 2.17, respectively. The highest concentrations of BUN in the calves were those from βC-treated, fourth-lactation cows at 15.41 mg/dL. Calves from the Con group cows had higher activities of GGT in serum than those from βC group cows. Table 2.18 shows the LS means by treatment and time point for GGT. Activities of GGT were highest at 24 h for calves from both groups at 983.3 U/L and 1508 U/L for βC and Con, respectively. The GGT activities were higher for calves from Con cows than for calves from βC cows at all time points.

Trends towards significance were found for BHBA (P =0.097) and phosphorus (P = 0.088), with phosphorus the only one o found to be non-significant for the interaction of treatment and parity. A number of variables that had no treatment effect had an interaction of treatment and parity, which are also shown in the table. There was a significant parity effect on BUN (P = 0.036), calcium (P < 0.01), and sodium (P = 0.024). There was significance or trend for treatment by parity interaction for BUN and sodium, which confounds the parity effect for those variables.

Discussion

The goal of this trial was to determine the effects of β-carotene supplementation on the cow, colostrum, and calf using cows with adequate vitamin A and E status from a well-managed herd. While much research has been done supplementing β-carotene to cows, some studies expected β-carotene to be used as provitamin A, while another assumed it had non-provitamin A effects without accounting for the baseline vitamin status of the animals (Oliveira et al., 2015). Because of this, our study made a point of analyzing the vitamin status of the feedstuffs and the serum of both cows and calves.

The vitamin E values in the TMR were slightly under the NRC (2001) requirements of 1,211 IU/d and 545 IU/d for the close-up and fresh groups, respectively. Compared to the NRC requirements for vitamin A of 69,850 IU/d for both groups, the close-up ration was within recommended levels at 84,460 IU/d while the fresh ration was remarkably high at 263,200 IU/d. This amount of vitamin A in the fresh ration was 46% higher than the formulated amount of 180,000 IU/d. The high values in the fresh cow ration were quite possibly a result of sampling error as only 2 wk of samples were taken and composited into one sample for analysis. While the formulated amounts of vitamin A for both groups were quite high, it was far from the amount

necessary to cause hypervitaminosis A (NRC, 2001; Combs, 2008). The adequate to high amounts of vitamin A fed should have allowed β-carotene to maximize its role as an antioxidant as it should not have been required as provitamin A. However, even with the dietary vitamin A exceeding the NRC (2001) requirements, serum retinol concentrations were increased by β-carotene supplementation. This significant treatment effect indicates that some β-carotene was still used as provitamin A, supporting the premise that the NRC (2001) levels for vitamin A should be increased (DSM, 2011). This finding also highlights that serum vitamin concentrations should be checked in every β-carotene research study before drawing conclusions on the effects of β-carotene.

Even though some β-carotene evidently was converted to retinol in the small intestine, β-carotene concentrations in serum of the cows was still significantly increased from an overall mean of 0.71 μg/mL in control cows to 2.93 μg/mL in supplemented cows. The β-carotene status of the herd at the beginning of the trial was quite low at 0.85 μg/mL. Cows supplemented with β-carotene achieved a peak serum concentration of 3.64 μg/mL, while the cows who were not supplemented peaked at 0.86 μg/mL. β-Carotene supplementation did not affect vitamin E serum concentrations. Vitamin E concentrations in the diet and cow serum were adequate; perhaps an interrelationship between β-carotene and vitamin E would have been seen if this was not the case.

Total protein, albumin, globulin, and albumin:globulin were all affected by βC supplementation. The concentration of TP was higher in βC cows at 6.73 g/dL compared to 6.50 g/dL for Con cows. While there was a treatment by parity interaction for all but TP, we observed that, overall, supplementation of β-carotene decreased the amount of albumin and increased the amount of globulin. This decrease in albumin is puzzling. It could mean the cow has less

potential for transporting nutrients throughout the body. Alternately, it could mean the cow had less of a need for aforementioned transport, meaning she could have been in a more stable state during the transition period. Either way, the decrease in albumin concentration that is typically seen around parturition (Rowlands and Manston, 1983) was greater in the cows that received β-carotene. The increase in globulin concentrations is interesting and agrees with past literature. Chew and Park (2004) showed that β-carotene had an immunostimulatory effect, meaning it can cause an elevated amount of globulins in circulation.

The effects on CPK are interesting. Creatine phosphokinase is an intriguing enzyme that is important for energetics. Elevated activitiess of CPK are often thought of as unfavorable as they may indicate severe breakdown of muscle tissue. Alternately, they could be high even in healthy animals who are experiencing high levels of metabolic activity, especially activity involving ATPases (Wallimann et al., 1992; Wallimann and Hemmer, 1994). When viewed this way, it may be possible that the elevated activities of CPK allowed the cow to be more efficient at utilizing energy, thus also aiding her in achieving a positive energy balance. The levels were higher at -7 d and 0 d for Con cows when compared to βC cows, but at 7 d, the βC cows had the higher concentration. The implications of these effects are unclear and bear further investigation.

Lack of difference in the number of days dry between treatment groups indicates that there was no difference in vitamin or metabolite status due to days dry. The lack of treatment response when investigating the health data may not be surprising as the power analysis for sample size was conducted on the basis of blood vitamin concentrations. A much greater sample size is generally needed to detect health differences.

While the results gleaned from the colostrum data mostly fell in line with previously published literature, our results prove the generally held supposition that β-carotene is associated with colostrum color. β-Carotene-supplemented cows had values of 2.40 and 28.30 for a* and b* color parameters, respectively, while control cows had values of -0.11 and 23.83. This means that β-carotene-rich colostrum was more red and yellow than the colostrum from control cows. Other carotenoids most likely contribute to colostrum color as well and should be investigated further. It is important to emphasize that IgG and BRIX values were only slightly to moderately correlated with colostrum color, so color was not a good indicator of colostrum quality. Proper quality measurements should be made to only feed calves colostrum with an IgG concentration of >50 mg/mL or a BRIX score of >22% (Godden, 2008, Bielmann et al., 2010). While there was not a treatment effect on IgG or BRIX, colostrum overall was of high quality, averaging 78.3 mg/mL and 25.1% for IgG and BRIX, respectively.

Another interesting point from the colostrum data is that β-carotene-treated cows had significantly increased colostrum fat percentage from 3.47% in the control cows to 4.41% in the supplemented cows. While not a direct economic effect for dairy producers, they could benefit from improved nutrients for the neonate. This increased amount of fat in the colostrum could indicate that the cow was in a more positive energy balance and could partition more energy towards colostrum production. It would be interesting to determine if this fat increase persists in milk, as well as if it changes the lipid profile of the milk.

β-Carotene concentrations in serum of the calves were extremely low at all time points, with the highest single value being 0.23 μg/mL. The β-carotene benefit gained from colostrum peaked at 24 h, was greatly diminished by d 7, and non-existent at d 60. This conflicts with the recommendation given by Iwanska et al. (1986) that the best way to increase calf serum β-

carotene concentrations is by supplementing the dam. There were no significant independent treatment effects on the metabolites of the calf. There were significant treatment effects for BUN and GGT, both of which also had a strong trend towards a treatment by parity interaction. Mean BUN was higher in calves from βC-treated dams entering their fourth lactation. Higher BUN can indicate kidney stress, excess protein in circulation, or dehydration. Lower GGT in calves from βC-treated dams for all lactations may indicate the liver is operating more efficiently or that it is less stressed. To unequivocally determine effects of increased β-carotene on calves, further research should investigate the effects of direct supplementation of β-carotene to calves.

Conclusion

While much research has been done on β-carotene, there were still many remaining questions and conflicting results regarding the effects of β-carotene, along with its interactions with vitamins A and E. The data collected during this study add to the pool of knowledge on the subject and suggests further research goals. The first question asked for this trial was regarding the effects of β-carotene on the cow. β-Carotene supplementation during the closeup period significantly increased β-carotene and vitamin A concentrations in the serum, but did not affect vitamin E. It decreased albumin and increased globulin, which all could indicate favorable responses for the cow in restoring energy balance, but these results were confounded by a treatment by parity interaction. Supplementation of βC independently increased TP and tended to decrease chloride. When looking at the effect of β-carotene supplementation on colostrum, an increase in a* and b* was seen, indicating that β-carotene caused colostrum to be more red and yellow. Colostrum from cows supplemented with β-carotene also had increased amounts of β-carotene and fat, but supplementation did not affect IgG content. Lastly, the supplementation of β-carotene to the dam had little effect on the calf. While calves from cows that received β-

carotene were more likely to have detectable serum concentrations of β-carotene at 24 h and d 7, the response did not last. There was either a significant treatment effect or a tendency towards significance for BHBA, BUN, phosphorus, and GGT in the calf serum; however, only phosphorus was independent of a treatment and parity or treatment by time interaction. Thus, β-carotene supplementation of the cow did not make a momentous change on the calf and direct supplementation should be investigated. Overall, prepartum β-carotene supplementation of cows had interesting effects on the cow and colostrum, but not on the calf.

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Tables and Figures

Table 2.1. Mean descriptive values by treatment group.

	Treatment LS Means			*P*-values
Variable	Con	βC	SE	Trt
Parity	2.40	2.45	0.092	0.75
Previous lactation milk yield, kg	12,880	12,340	839	0.32
Days dry	52.8	52.3	0.65	0.60

Table 2.2. Formulated diet for close-up dry multiparous cows.[1]

Ingredient	As-fed (kg/head/d)	Dry matter (kg/head/d)
Corn silage	11.1	3.70
Water	3.63	-
Straw	2.74	2.36
Canola meal	2.09	1.87
Prairie grass hay	1.60	1.38
Premix	1.55	1.41
Ground corn	0.930	0.816
Pushout	0.894	0.454
Hay, dry	0.830	0.703
Total	25.37	12.7

[1] Formulated diets for far-off dry multiparous cows and fresh cows are available in the Appendix.

Table 2.3. Feed sample analysis for close-up dry multiparous cows TMR, wk 1-3.[1,2]

Components	Unit	As-fed	Dry matter
Moisture	%	49.7	
Dry matter	%	50.3	
Crude protein	%	8.00	15.9
Available protein	%	7.40	14.6
ADICP	%	0.60	1.30
Adjusted crude protein	%	8.00	15.9
Soluble protein	% CP		33.0
NDICP	%	1.50	2.90
ADF	%	15.3	30.5
aNDFom	%	22.8	45.4
Lignin	%	2.80	5.60
NFC	%	13.0	25.9
Starch	%	8.00	15.9
ESC (Simple sugars)	%	1.70	3.30
Crude fat	%	1.70	3.40
Ash	%	4.73	9.39
TDN	%	31	62
NEl	Mcal/kg	0.71	1.4
NEm	Mcal/kg	0.66	1.3
NEg	Mcal/kg	0.38	0.75
Calcium	%	0.61	1.2
Phosphoros	%	0.17	0.35
Magnesium	%	0.24	0.47
Potassium	%	0.58	1.2
Sodium	%	0.043	0.085
Iron	ppm	350	690
Zinc	ppm	63	130
Copper	ppm	16	31
Manganese	ppm	73	150
Molybdenum	ppm	0.90	1.7
Sulfur	%	0.22	0.44
Choloride ion	%	0.43	0.85
DCAD	mEq/100g	-	-18

[1] Feed sample analysis for fresh cow diet is available in the Appendix.
[2]Weekly samples from the first 3 weeks of trial were composited into one sample for analysis.

Table 2.4. Feed sample analysis for close-up dry multiparous cows TMR, wk 4-6.[1,2]

Components	Unit	As-fed	Dry matter
Moisture	%	52.0	
Dry matter	%	48.0	
Crude protein	%	7.30	15.3
Available protein	%	6.80	14.3
ADICP	%	0.50	1.00
Adjusted crude protein	%	7.30	15.3
Soluble protein	% CP		35.0
NDICP	%	1.30	2.70
ADF	%	13.6	28.4
aNDFom	%	19.9	14.5
Lignin	%	2.40	5.00
NFC	%	14.7	30.7
Starch	%	9.80	20.4
ESC (Simple sugars)	%	2.80	5.90
Crude fat	%	1.70	3.50
Ash	%	4.40	9.16
TDN	%	31	64
NEl	Mcal/kg	0.71	1.5
NEm	Mcal/kg	0.68	1.4
NEg	Mcal/kg	0.40	0.84
Calcium	%	0.64	1.34
Phosphoros	%	0.16	0.33
Magnesium	%	0.23	0.48
Potassium	%	0.49	1.02
Sodium	%	0.045	0.094
Iron	ppm	360	760
Zinc	ppm	59	120
Copper	ppm	14	29
Manganese	ppm	69	140
Molybdenum	ppm	0.50	1.1
Sulfur	%	0.20	0.42
Choloride ion	%	0.44	0.92
DCAD	mEq/100g		-22

[1] Feed sample analysis for fresh cow diet is available in the Appendix.
[2]Weekly samples from the second 3 weeks of trial were composited into one sample for analysis.

Table 2.5. Vitamin concentrations in sampled feedstuffs.

Sample description	β-Carotene in feed (μg/g)	Mean (μg/g)	Vit E activity in feed (IU/kg)	Mean (IU/kg)	α-Tocopherol in feed (μg/g)	Mean (μg/g)	Vit A activity in feed (IU/kg)	Mean (IU/kg)	Retinol in feed (ng/g)	Mean (ng/g)
Close-up										
Silage wk 1-3	5.31	4.41	0.6	0.5	0.57	0.48	-[1]	-	-	-
Silage wk 4-6	3.50		0.4		0.39		-	-	-	-
Hay wk 1-3	5.87	3.87	5.2	5.15	4.75	4.68	-	-	-	-
Hay wk 4-6	1.86		5.1		4.60		-	-	-	-
Pre-mix	0.49	0.49	54.6	54.6	49.7	49.7	7060	7060	2120	2120
TMR wk 1-3	0.83	0.81	164	87.8	149	79.8	7270	6650	2180	2000
TMR wk 4-6	0.79		12.1		11.0		6030		1810	
Fresh										
Silage	5.13	5.13	0.5	0.5	0.42	0.42	-	-	-	-
TMR	1.64	1.64	28.0	28.0	25.4	25.4	14500	14500	4366	4366

[1] Note: corn silage and hay not tested for vitamin A due to no pre-formed retinol being found in forages.

Table 2.6. Vitamins A and E NRC (2001) requirements, DSM (2011) recommendations, formulated amounts in TMR, and actual amounts in TMR.

	Vitamin E					Vitamin A				
Sample description	NRC values[1] (IU/d)	DSM values[2] (IU/d)	Formulated (IU/d)	Actual (IU/d)	Actual mean (IU/d)	NRC values[1] (IU/d)	DSM values[2] (IU/d)	Formulated (IU/d)	Actual (IU/d)	Actual mean (IU/d)
Close-up[3]										
TMR wk 1-3	1210	1110 to 3330	1250	2080	1116	69,900	75,000 to 100,000	317000	92,300	84,500
TMR wk 4-6				154					76,600	
Fresh[4]										
TMR	545	1110 to 3330	754	507	507	69,900	75,000 to 100,000	181,000	263,000	263,000

[1]Requirements (NRC, 2001).

[2]Recommendations (DSM, 2011).

[3] Total DM fed was 12.7 kg of DM/d.

[4]Total DM fed was 18.1 kg of DM/d.

Table 2.7. Results of cow serum sample analysis.

Variable	Treatment LS Means[1]			*P*-values					
	Con	βC	SE	Trt	Parity	Trt* Parity	Pen	Day	Trt* Day
Vitamins									
β-Carotene, μg/mL	0.71	2.95	0.13	<0.01	0.94	0.34	0.72	<0.01	<0.01
Vit A, ng/mL	144.2	163.2	6.3	0.023	<0.01	0.99	0.068	<0.01	0.14
Vit E, μg/mL	2.301	2.182	0.11	0.26	<0.01	0.46	0.47	<0.01	0.28
Metabolites									
NEFA, mmol/L	0.555	0.548	0.068	0.90	0.10	0.41	0.77	<0.01	0.70
BHBA, μmol/L	489	478	40	0.77	<0.01	0.88	0.74	<0.01	0.43
Creatinine, mg/dL	0.99	0.96	0.022	0.29	0.62	0.14	0.59	<0.01	0.64
BUN, mg/dL	14.0	13.5	0.51	0.33	0.36	0.29	0.70	<0.01	0.94
TP, g/dL	6.50	6.73	0.11	0.045	<0.01	0.33	0.11	<0.01	0.60
Albumin, g/dL	3.19	3.06	0.054	0.029	0.84	<0.01	0.76	<0.01	0.54
Globulin, g/dL	3.27	3.55	0.10	<0.01	0.20	0.07	0.13	<0.01	0.89
Albumin:Globulin	0.99	0.87	0.027	<0.01	<0.01	<0.01	0.15	<0.01	0.76
Calcium, mg/dL[3]	8.84	8.87	0.089	0.75	0.19	0.43	0.83	<0.01	0.41
P, mg/dL	4.71	4.85	0.12	0.38	0.24	<0.01	0.99	<0.01	0.09
Na, mmol/L	139.7	138.0	1.02	0.12	0.54	0.18	0.98	<0.01	0.46
K, mmol/L[3]	4.35	4.34	0.052	0.88	0.042	0.90	0.46	<0.01	0.53
Na:K	32.3	32.6	0.35	0.61	0.01	0.45	0.45	<0.01	0.78
Chloride, mmol/L	104.9	103.4	0.92	0.080	0.58	0.10	0.75	<0.01	0.66
Glucose, mg/dL	68.0	69.0	3.7	0.76	0.076	0.88	0.42	<0.01	0.60
Alkaline phosphatase, U/L	42.8	41.6	1.5	0.54	0.96	0.59	0.65	0.01	0.97
AST, U/L	79.6	73.6	4.6	0.34	0.82	0.92	0.82	<0.01	0.42
GGT, U/L	16.2[2]	17.4[2]	0.87[2]	0.29	0.47	0.07	0.89	0.63	0.64
Bilirubin, mg/dL[4]	-	-	-	-	-	-	-	-	-
CPK, U/L	202.0	143.0	49.6	0.21	0.43	0.37	0.84	0.16	0.03

Table 2.7 (cont.)

Variable	Treatment LS Means[1]			*P*-values					
	Con	βC	SE	Trt	Parity	Trt* Parity	Pen	Day	Trt* Day
Cholesterol, mg/dL	91.2	95.8	3.6	0.19	<0.01	0.061	0.070	<0.01	0.91
GLDH, U/L	42.1[2]	44.3[2]	5.6[2]	0.75	<0.01	0.69	0.23	0.071	0.37
Bicarb, mmol/L	18.7	18.9	0.42	0.63	<0.01	0.42	0.19	<0.01	0.49
Magnesium, mg/dL	2.1	2.0	0.04	0.25	0.79	0.035	0.89	<0.01	0.59
Triglycerides, mg/dL	17.7	17.7	1.0	0.99	0.89	0.59	0.99	<0.01	0.64
Anion gap, mg/dL	20.6	20.6	0.63	0.92	0.61	0.66	0.90	<0.01	0.76

[1]Means are from d -7, d 0, and d 7.

[2]From non-transformed data.

[3]Used unstructured covariance structure.

[4]Model failed to converge.

Table 2.8. Cow health results for continuous variables.

	Treatment LS Means			*P*-values			
Variable	Con	βC	SE	Trt	Parity	Trt* Parity	Pen
Days dry	52.77	52.28	0.65	0.32	<0.01	0.16	0.11
Days to conception[1]	99.57	102.3	18	0.90	0.39	0.96	0.099
Times bred[1]	2.275	2.856	0.48	0.38	0.45	0.79	0.16
DHI 305ME[1,2],kg	15,630	15,430	660	0.81	0.12	0.84	0.80
DairyComp 305ME[1,3],kg	13,840	13,550	560	0.69	0.97	0.89	0.63
Fresh milk yield[4],kg/d	48.9	46.5	2.3	0.41	0.026	0.69	0.13
BCS decrease	0.22	0.30	0.088	0.53	0.46	0.74	0.84

[1]Data may have changed since last DairyComp 305 backup on March 8, 2016.

[2]Dairy Herd Improvement calculated 305 d mature equivalent.

[3]DairyComp calculated 305 d mature equivalent.

[4]Milk yield at 30 DIM.

Table 2.9. Cow health results for binomial variables.

Variable[12]	Level	Coefficient	SE	OR[1]	95% CL	*P*- value
Pregnant[2,4]	Confirmed pregnant	0.139	0.46	1.1	0.46-2.9	0.76
Sold[3,5]	Left the herd	-0.315	0.95	0.73	0.11-4.8	0.74
DA[3,6]	DA incidence	-0.0002	0.74	1.0	0.23-4.3	0.99
Off feed[3,7]	Went off feed	1.143	1.2	3.1	0.31-32	0.33
Lame[3,8]	Lameness incidence	0.989	0.86	2.7	0.48-15	0.26
Footrot[3,9]	Footrot incidence	-0.311	0.79	0.73	0.15-3.5	0.70
Mastitis[3,10]	Clinical mastitis incidence	-0.77	1.2	0.46	0.039-5.5	0.54
Metritis[3,11]	Metritis incidence	0.425	0.94	1.5	0.24-9.8	0.65

[1]Odds ratio for βC cows compared to Con cows.

[2]As of 120 DIM.

[3]As of 30 DIM.

[4]BC [n = 39; not pregnant (referent) = 15, pregnant = 24], Con [n = 42; not pregnant (referent) = 18, pregnant = 24], not bred [n = 13].

[5]BC [n = 47; remained in herd (referent) = 45, left the herd = 2] and Con [n = 47; remained in herd (referent) = 44, left the herd = 3].

[6]BC [n = 47; healthy (referent) = 43, DA incidence = 4] and Con [n = 47; healthy (referent) = 43, DA incidence = 4].

[7]BC [n = 47; healthy (referent) = 44, went off feed = 3] and Con [n = 47; healthy (referent) = 46, went off feed = 1].

[8]BC [n = 47; healthy (referent) = 42, lameness incidence = 5] and Con [n = 47; healthy (referent) = 45, lameness incidence = 2].

[9]BC [n = 47; healthy (referent) = 44, footrot incidence = 3] and Con [n = 47; healthy (referent) = 43, footrot incidence = 4].

[10]BC [n = 47; healthy (referent) = 46, DA incidence = 1] and Con [n = 47; healthy (referent) = 45, DA incidence = 2].

[11]BC [n = 47; healthy (referent) = 44, DA incidence = 3] and Con [n = 47; healthy (referent) = 45, DA incidence = 2].

[12]Ketosis, milk fever, and retained placentas were excluded because not enough incidents occurred to perform statistical analysis.

Table 2.10. Results of colorimetry, quality, vitamin, and component analysis of colostrum.

Variable	Treatment LS Means			*P*-values			
	Con	βC	SE	Trt	Parity	Trt* Parity	Pen
Vitamins							
β-carotene, μg/mL	0.64	1.53	0.12	<0.01	0.53	0.51	0.046
Vit A, ng/mL	4375	5071	620	0.25	0.017	0.31	0.61
Vit E, μg/mL	3.18	3.48	0.41	0.51	0.63	0.71	<0.01
Colorimeter							
L*	78.5	78.6	1.3	0.95	<0.01	0.11	0.79
a*	-0.11	2.40	0.84	0.014	0.55	0.45	0.044
b*[1]	23.8	28.3	1.3	<0.01	0.023	0.95	0.079
Quality							
IgG, mg/mL	86.0	89.0	5.2	0.55	<0.01	0.75	0.12
BRIX %	26.8	27.3	1.0	0.62	<0.01	0.76	0.46
Components							
Fat, %	3.472	4.414	0.47	0.042	0.25	0.085	0.43
Protein, %	16	15.80	0.50	0.77	0.22	0.62	0.95
SCC/mL × 1000	512.7	947.5	410.0	0.21	0.30	0.56	0.64
Lactose, %	2.28	2.48	0.14	0.29	0.24	0.066	0.56
Other solids, %	3.96	4.06	0.12	0.52	0.13	0.12	0.57
Total solids, %	23.11	22.67	0.69	0.65	0.56	0.10	0.76
MUN, mg/dL	38.6	35.3	2.4	0.33	0.59	0.66	0.95

[1]LS Mean values taken from non-transformed b* data. *P*-values taken from transformed data.

Table 2.11. Results for color score of colostrum.

Variable	Treatment LS Means			*P*-values			
	Con	βC	SE	Trt	Parity	Trt* Parity	Pen
Color score	2.40	3.23	0.18	<0.01	0.03	0.35	0.26

Table 2.12. Colostrum β-carotene concentrations by color score.

	Colostrum Color Score			
	1	2	3	4
Color chip				
Picture				
β-carotene, µg/mL	-[1]	0.56	0.93	1.72

[1]No cows on trial had colostrum with a color score of 1.

Table 2.13. Least squares (LS) means by treatment and parity for colostrum fat percentage.

Parity No.	LS Means		
	Con	βC	SE
2	3.61	3.45	0.47
3	3.28	4.46	0.58
4	3.53	5.34	0.89

Table 2.14. Correlations among vitamin A, vitamin E, β-carotene, and other variables in colostrum.[1]

	VitE	VitA	βC	BRIX	IgG	L*	a*	b*	Fat %	Protein %	SCC
VitE	1.0[2]	0.31	0.46	0.55	0.37	0.11	0.39	0.28	0.45	0.30	0.13
	--[2]	<0.01	<0.01	<0.01	<0.01	0.30	<0.01	<0.01	<0.01	<0.01	0.23
	93[2]	93	93	93	93	93	73	93	88	85	88
VitA	0.31	1.0	0.42	0.27	0.06	<0.01	0.32	0.23	0.34	0.029	-0.098
	<0.01	--	<0.01	0.01	0.54	0.98	<0.01	0.027	<0.01	0.79	0.368
	93	93	93	93	93	93	73	93	88	85	88
βC	0.46	0.42	1.0	0.28	0.18	-0.080	0.48	0.54	0.44	0.067	0.19
	<0.01	<0.01	--	<0.01	0.08	0.44	<0.01	<0.01	<0.01	0.54	0.082
	93	93	93	93	93	93	73	93	88	85	88

[1]Full colostrum correlation results shown in Table A5.

[2] Correlation

P-value

n

Table 2.15. Results of calf serum sample analysis.

Variable	Treatment LS Means			*P*-values				
	Con	βC	SE	Trt	Parity	Trt* Parity	Time	Trt* Time
Vitamins								
β-carotene, μg/mL[1]	-	-	-	-	-	-	-	-
Vit A, ng/mL	123.4	124.2	4.6	0.89	0.46	0.41	<0.01	0.24
Vit E, μg/mL	0.390	0.360	0.028	0.43	0.73	<0.01	<0.01	0.96
Metabolites								
NEFA, mmol/L[2]	0.483	0.386	0.051	0.17	0.35	0.032	<0.01	0.91
BHBA, μmol/L[3]	57.0	72.5	6.6	0.097	0.39	0.043	-	-
Creatinine, mg/dL	1.68	1.66	0.086	0.84	0.25	0.16	<0.01	0.73
BUN, mg/dL	12.23	13.23	0.36	0.041	0.036	0.057	<0.01	0.93
TP, g/dL	5.82	5.84	0.086	0.83	0.51	0.67	<0.01	0.39
Albumin, g/dL[4]	-	-	-	-	-	-	-	-
Globulin, g/dL	3.231	3.235	0.076	0.96	0.88	0.25	<0.01	0.62
Albumin:Globulin	0.99	0.96	0.018	0.31	0.40	0.030	<0.01	0.98
Calcium, mg/dL	10.79	10.97	0.10	0.17	0.0035	0.18	<0.01	0.24
P, mg/dL	7.11	7.39	0.13	0.088	0.36	0.32	<0.01	0.86
Na, mmol/L	136.1	136.3	0.93	0.87	0.024	<0.01	<0.01	0.13
K, mmol/L	5.07	5.19	0.15	0.56	0.31	0.88	<0.01	0.82
Na:K	26.9	27.0	0.58	0.94	0.89	0.59	<0.01	0.22
Chloride, mmol/L	96.7	96.3	0.66	0.65	0.17	0.010	<0.01	0.20
Glucose, mg/dL	74.2	80.6	3.7	0.18	0.26	0.47	<0.01	0.78
Alkaline phosphatase, U/L	237	229	15	0.65	0.12	0.079	<0.01	0.071
AST_SGOT, U/L	39.8	42.1	5.5	0.73	0.97	0.65	<0.01	0.19
GGT, U/L	458	276[5]	47[5]	<0.01	0.96	0.051	<0.01	<0.01
Bilirubin, mg/dL	0.17	0.69[5]	1.4[5]	0.91	0.19	0.20	<0.01	0.21
CPK_CK, U/L	187.9	217.5	18	0.22	0.90	0.20	<0.01	0.47

Table 2.15 (cont.)

	Treatment LS Means			*P*-values				
Variable	Con	βC	SE	Trt	Parity	Trt* Parity	Time	Trt* Time
Cholesterol, mg/dL	43.4	41.5	1.5	0.30	0.60	<0.01	<0.01	0.90
GLDH, U/L	37.0	24.6	6.4	0.14	0.69	0.83	<0.01	0.057
Bicarb, mmol/L	20.95	20.21	0.34	0.11	0.34	<0.01	<0.01	0.53
Magnesium, mg/dL[4]	-	-	-	-	-	-	-	-
Triglycerides, mg/dL	17.9	18.5	1.2	0.71	0.17	0.31	<0.01	0.011
Anion gap, mg/dL	23.9	24.7	0.40	0.12	0.69	0.15	<0.01	0.20

[1]Samples were mostly below the test threshold so entered as missing samples.

[2]Only 0 d and 24 h were tested.

[3]Only 0 d was tested.

[4]Could not get model to converge.

[5]From non-transformed data.

Table 2.16. Least squares (LS) means by treatment and parity for calf serum BUN (mg/dL).

Parity No.	LS Means		
	βC	Con	SE
2	12.26	12.27	0.32
3	12.01	12.12	0.48
4	15.41	12.31	0.98

Table 2.17. Least squares (LS) means by treatment and parity for calf serum GGT (U/L).

Parity No.	LS Means		
	βC	Con	SE
2	316.01	428.23	40
3	313.46	432.12	61
4	197.42	511.50	126

Table 2.18. Least squares (LS) means by treatment and time point for calf serum GGT (U/L).

Time Point	LS Means		
	βC	Con	SE
0	-23.17	30.02	67
24	983.3	1508	72
7	158.3	250.4	67
60	-15.85	40.85	73

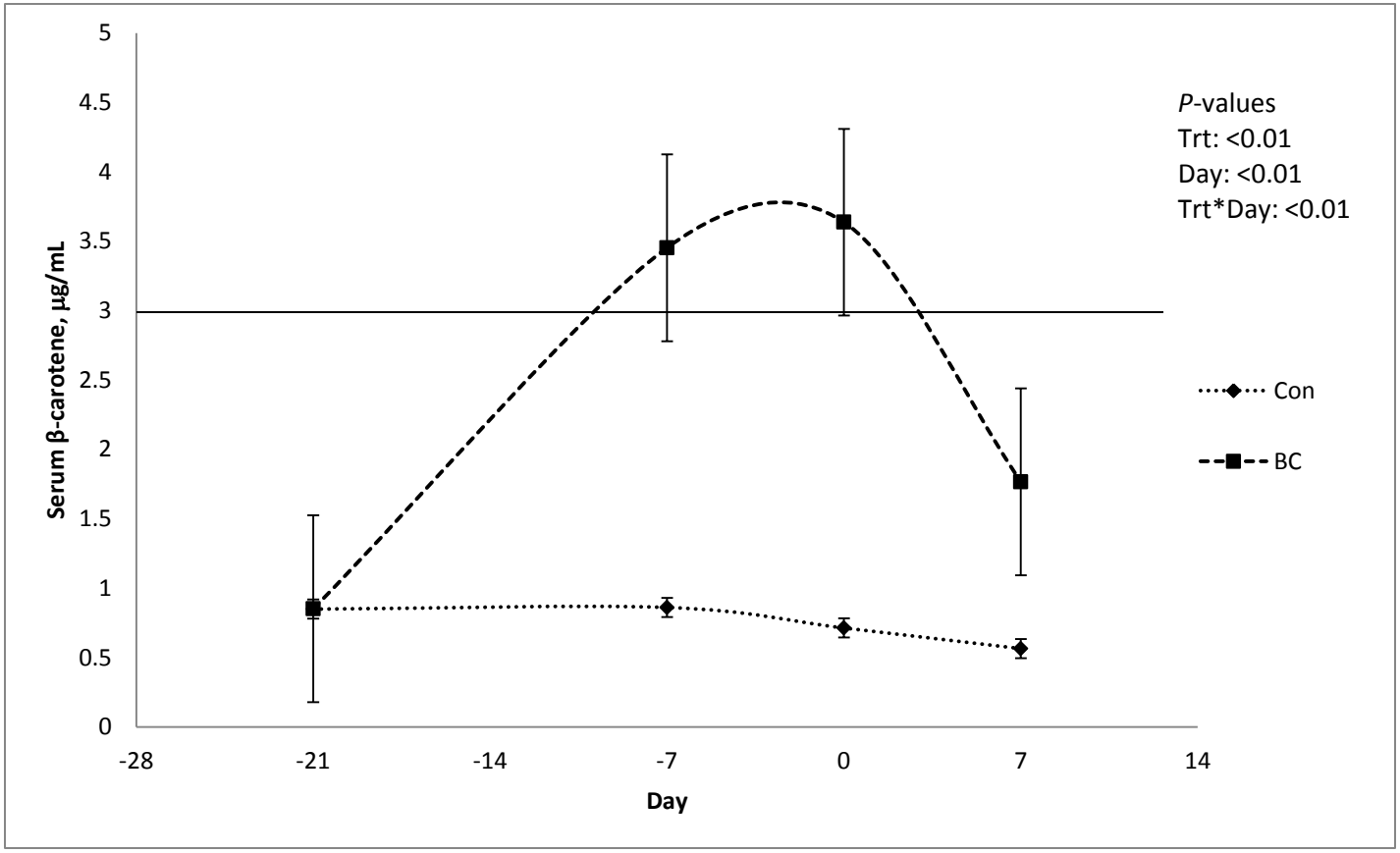

Figure 2.1. Cow serum β-carotene (µg/mL) concentrations by day with guideline cutoff shown at 3 µg/ml.

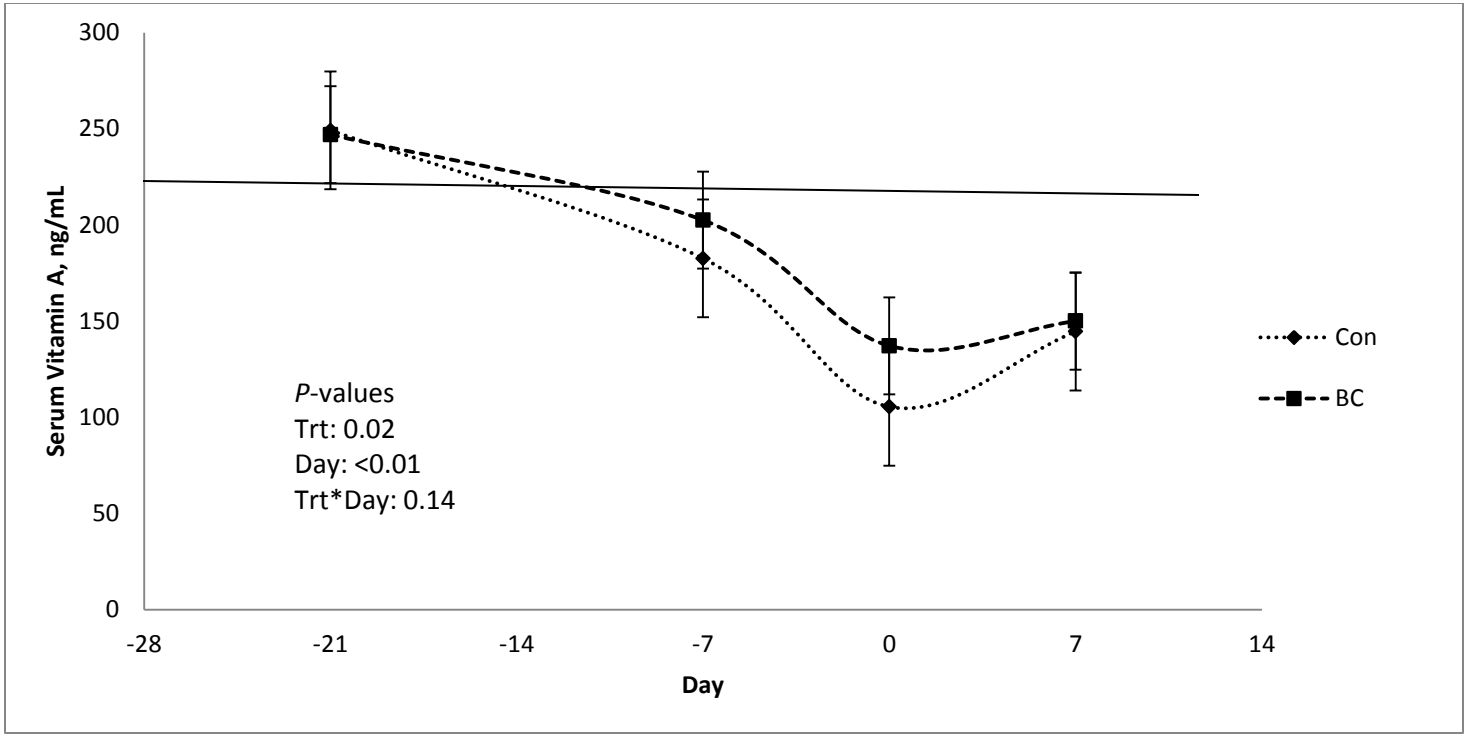

Figure 2.2. Cow serum vitamin A (ng/mL) concentrations by day with guideline cutoff shown at 250 ng/ml.

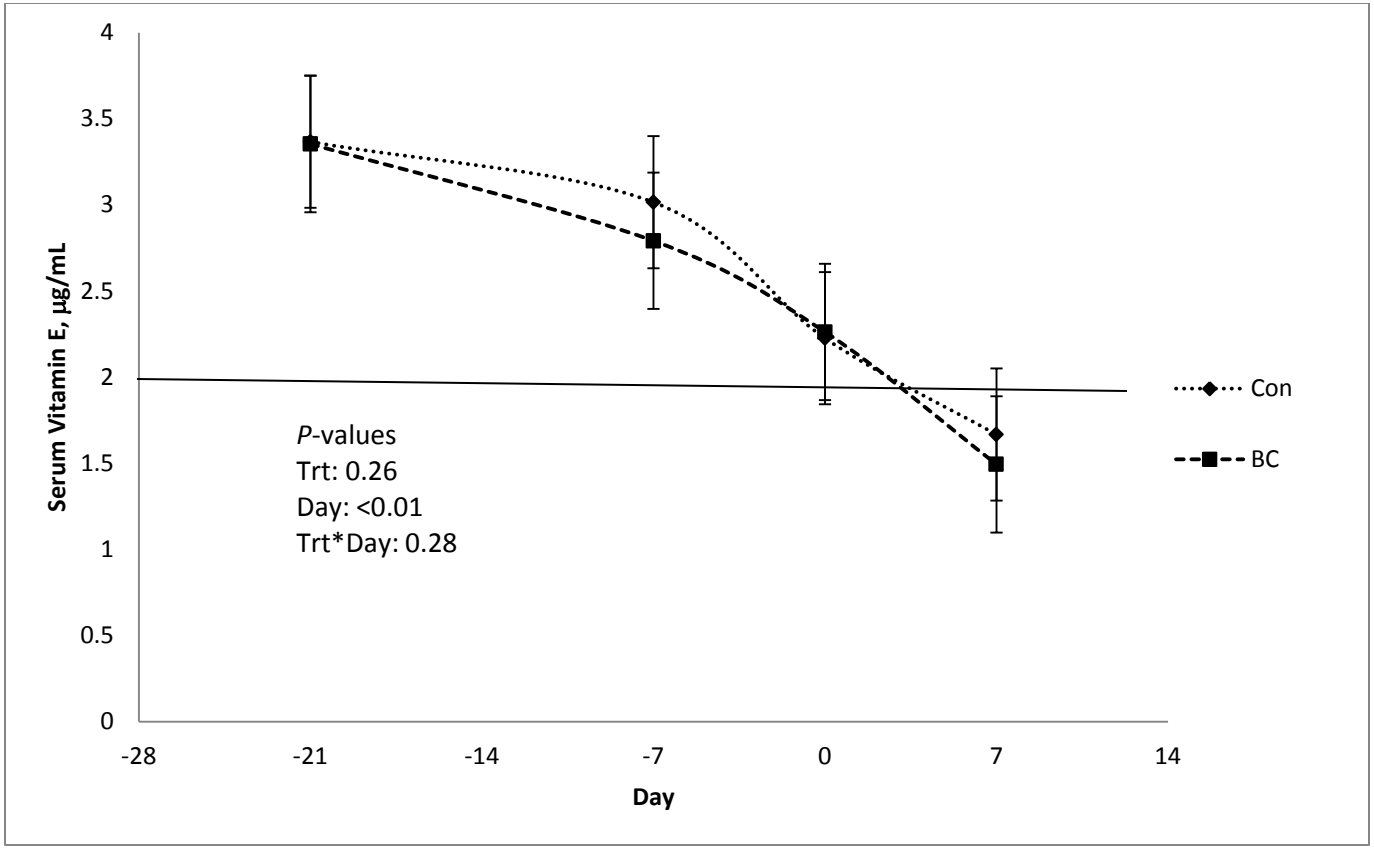

Figure 2.3. Cow serum vitamin E (µg/mL) concentrations by day with guideline cutoff shown at 2 µ/ml.

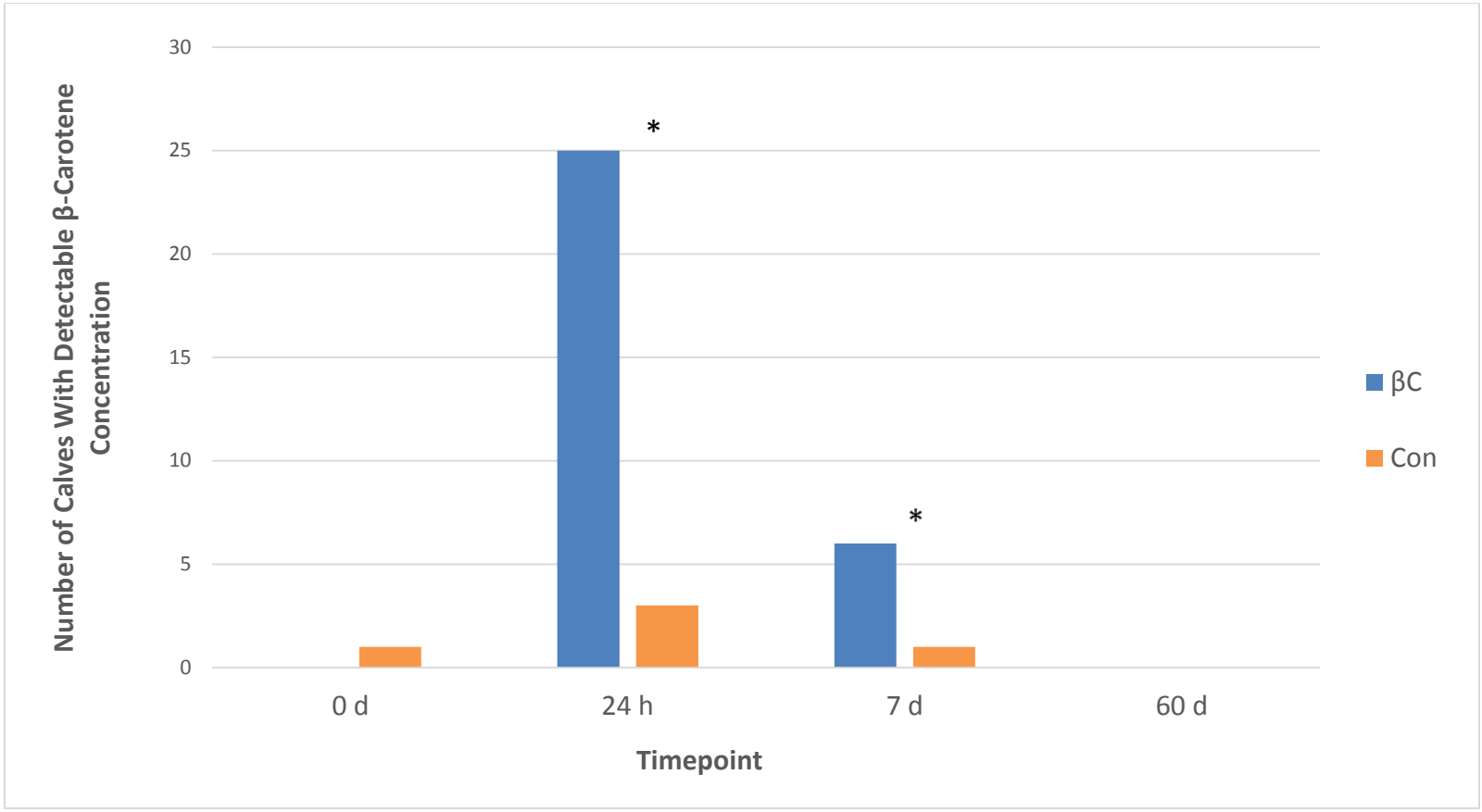

Figure 2.4. Number of calves with detectable[1] β-carotene concentration.

[1]Levels were detectable at ≥ 0.05 µg/mL.

*Significantly different from Con.

CONCLUDING REMARKS

Due to changing consumer demands, it is important that the dairy industry fine-tunes their practices. A collective goal of the evolving dairy industry is to raise healthy and productive animals while avoiding the use of drugs as much as possible and maintaining profitability for the producer. One way of working towards accomplishing this is to utilize antioxidants in the diets of dairy cattle. β-Carotene has long been recognized for its provitamin A role, but in the past few decades has been gaining a reputation separate from vitamin A as a potent antioxidant. Including β-carotene in the ration of dairy cows can provide a source of extra vitamin A in times of need and should thus be considered when formulating the ration. Additionally, increased β-carotene in the serum may lead to favorable changes in metabolites, such as increasing total protein and globulin. Supplementation of β-carotene to cows increased the amount of β-carotene in the colostrum. It also raised the fat percentage and caused the color markers of a* and b* to be higher, meaning the colostrum was more red-yellow. Importantly, however, this did not indicate any difference in colostral IgG content. Not many benefits were seen in the calves, although the number of calves with detectable concentrations of β-carotene was higher when there dams were treated with β-carotene. This response did not last long in the calf, indicating that improving the β-carotene status of the dam does not necessarily improve that of the calf. Direct β-carotene supplementation through milk replacer and grain should be considered for young calves.

APPENDIX

Table A.1. Penn State particle separator results for close-up cow diets.

Week[1]	Sample	Upper, % [2]	Middle, % [2]	Lower, % [2]	Pan, % [2]
1	Pen 45	16.48	36.48	19.52	27.52
	Pen 45 refusals	18.01	35.35	15.73	30.91
	Pen 41	11.69	40.27	17.44	30.59
	Pen 41 refusals	14.99	42.12	16.15	26.74
2	Pen 45	11.24	35.15	18.04	35.57
	Pen 45 refusals	10.08	37.03	18.89	34.01
	Pen 41	9.87	37.50	17.76	34.87
	Pen 41 refusals	18.36	33.90	17.66	30.08
3	Pen 45	13.83	33.19	14.70	38.28
	Pen 45 refusals	15.40	32.74	15.07	36.79
	Pen 41	12.21	37.50	15.84	34.45
	Pen 41 refusals	22.18	32.97	14.72	30.13
4	Pen 45	15.78	30.68	18.31	35.23
	Pen 45 refusals	17.84	35.52	16.01	30.64
	Pen 41	10.02	33.90	18.34	37.75
	Pen 41 refusals	12.26	33.38	18.46	35.90
5	Pen 45	8.51	36.12	17.91	37.46
	Pen 45 refusals	10.22	42.40	18.14	29.25
	Pen 41	12.80	35.27	17.63	34.30
	Pen 41 refusals	12.81	37.90	19.02	30.27
6	Pen 45	14.14	32.11	15.64	38.10
	Pen 45 refusals	15.07	36.81	17.10	31.01
	Pen 41	18.96	31.11	15.35	34.58
	Pen 41 refusals	8.87	42.86	16.13	32.14
	Average	**13.79**	**36.09**	**17.11**	**33.01**
	Recommended[3]	2-8	30-50	10-20	30-40

[1]Week of trial

[2]Amounts shown are material retained on sieve.

[3]Recommendations from Penn State for high-producing cows.

Table A.2. Penn State particle separator results for fresh cow diets.

Week[1]	Sample	Upper, % [2]	Middle, % [2]	Lower, % [2]	Pan, % [2]
5	Pen 8	8.41	31.93	16.63	43.02
	Pen 8 refusals	6.66	30.70	17.48	45.16
	Pen 28	6.44	35.12	14.93	43.51
	Pen 28 refusals	5.73	38.45	15.05	40.78
6	Pen 8	7.11	33.55	16.35	42.99
	Pen 8 refusals	7.38	31.40	18.88	42.34
	Pen 28	4.64	31.36	20.09	43.91
	Pen 28 refusals	6.12	37.73	18.22	37.93
	Average	**6.52**	**33.97**	**17.29**	**42.21**
	Recommended[3]	2-8	30-50	10-20	30-40

[1]Week of trial

[2]Amounts shown are material retained on sieve.

[3]Recommendations from Penn State for high-producing cows.

Table A.3. Visual descriptions of scores given for topdress consumption.

Topdress consumption scores				
0	1	2	3	4
Did not consume any	Did not consume a substantial amount, but exhibited licking behavior	Consumed roughly half	Consumed almost all	Consumed completely

Table A.4. Formulated diet for far-off dry multiparous cows.

Ingredient	As-fed (kg/head/d)	Dry matter (kg/head/d)
Corn silage	12.1	4.23
Water	4.08	-
Straw	3.96	3.40
Pushout	2.68	1.36
Prairie hay	1.85	1.59
Canola meal	1.66	1.50
Premix	0.177	0.168
Total	26.5	12.2

Table A.5. Formulated diet for fresh cows.

Ingredient	As-fed (kg/head/d)	Dry matter (kg/head/d)
Corn silage	15.6	5.22
Water	4.54	-
Ground corn	3.09	2.72
Haylage	2.39	0.839
Canola Meal	1.89	1.70
Baylage	1.86	1.16
Premix	1.51	1.43
Hominy	1.36	1.22
Ryelage	1.26	0.454
Cottonseed	0.962	0.839
Corn gluten meal	0.807	0.726
Soybean meal	0.757	0.680
Marshmallow	0.680	0.590
Hay, western	0.658	0.567
Total	37.3	18.1

Table A.6. Feed sample analysis for fresh cow TMR.

Components	Unit	As-fed	Dry matter
Moisture	%	50.7	
Dry matter	%	49.3	
Crude protein	%	8.10	16.4
Available protein	%	7.60	15.4
ADICP	%	0.50	1.00
Adjusted crude protein	%	8.10	16.4
Soluble protein	% CP		38.0
NDICP	%	1.30	2.60
ADF	%	11.2	22.7
aNDFom	%	15.8	32.2
Lignin	%	2.10	4.20
NFC	%	18.7	38.0
Starch	%	11.2	22.7
ESC (Simple sugars)	%	2.20	4.50
Crude fat	%	2.50	5.00
Ash	%	4.19	8.51
TDN	%	35	71
NEL	Mcal/kg	0.82	1.7
NEM	Mcal/kg	0.84	1.7
NEG	Mcal/kg	0.53	1.1
Calcium	%	0.43	0.87
Phosphoros	%	0.20	0.41
Magnesium	%	0.19	0.38
Potassium	%	0.75	1.5
Sodium	%	0.26	0.53
Iron	ppm	180	360
Zinc	ppm	130	260
Copper	ppm	14	29
Manganese	ppm	71	150
Molybdenum	ppm	0.70	1.4
Sulfur	%	0.14	0.28
Chloride ion	%	0.29	0.58
DCAD	mEq/100g		28